

SUPPLEMENTAL MATERIAL

Table-1 RNA-Seq analysis of upregulated genes in miR106b-93-25-/ ischemic-muscle vs. WT ischemic muscle

gene	sample_1	sample_2	log2(fold_change)	p_value	q_value	significant
Cxcl2	WT-IGA	miR106b-93-25-/ IGA	5.64515	5.00E-05	0.0135782	yes
Irg1	WT-IGA	miR106b-93-25-/ IGA	4.83218	0.0002	0.0423541	yes
Gm3893	WT-IGA	miR106b-93-25-/ IGA	3.8408	5.00E-05	0.0135782	yes
Ppbp	WT-IGA	miR106b-93-25-/ IGA	3.7507	5.00E-05	0.0135782	yes
Il1b	WT-IGA	miR106b-93-25-/ IGA	3.52656	5.00E-05	0.0135782	yes
Cxcl13	WT-IGA	miR106b-93-25-/ IGA	3.46558	5.00E-05	0.0135782	yes
Hdc	WT-IGA	miR106b-93-25-/ IGA	3.11125	5.00E-05	0.0135782	yes
Rn45s	WT-IGA	miR106b-93-25-/ IGA	3.07486	5.00E-05	0.0135782	yes
Ptgs2	WT-IGA	miR106b-93-25-/ IGA	3.07467	5.00E-05	0.0135782	yes
Nlrp12	WT-IGA	miR106b-93-25-/ IGA	2.78856	5.00E-05	0.0135782	yes
Cxcl1	WT-IGA	miR106b-93-25-/ IGA	2.77217	5.00E-05	0.0135782	yes
Fpr1	WT-IGA	miR106b-93-25-/ IGA	2.70597	5.00E-05	0.0135782	yes
Shh	WT-IGA	miR106b-93-25-/ IGA	2.60222	5.00E-05	0.0135782	yes
Trem1	WT-IGA	miR106b-93-25-/ IGA	2.59091	5.00E-05	0.0135782	yes
Inhbb	WT-IGA	miR106b-93-25-/ IGA	2.50146	5.00E-05	0.0135782	yes
Inmt	WT-IGA	miR106b-93-25-/ IGA	2.45125	0.00015	0.0342817	yes
Hspa1b	WT-IGA	miR106b-93-25-/ IGA	2.44436	5.00E-05	0.0135782	yes
Lrg1	WT-IGA	miR106b-93-25-/ IGA	2.31776	5.00E-05	0.0135782	yes
Saa3	WT-IGA	miR106b-93-25-/ IGA	2.27333	5.00E-05	0.0135782	yes
Mxd1	WT-IGA	miR106b-93-25-/ IGA	2.24413	5.00E-05	0.0135782	yes
Cish	WT-IGA	miR106b-93-25-/ IGA	2.21906	5.00E-05	0.0135782	yes
Osm	WT-IGA	miR106b-93-25-/ IGA	2.20738	5.00E-05	0.0135782	yes
Slfn4	WT-IGA	miR106b-93-25-/ IGA	2.16415	5.00E-05	0.0135782	yes
Hspa1a	WT-IGA	miR106b-93-25-/ IGA	2.15179	5.00E-05	0.0135782	yes
Draxin	WT-IGA	miR106b-93-25-/ IGA	2.14362	0.0001	0.0248204	yes
Ier2	WT-IGA	miR106b-93-25-/ IGA	2.13762	5.00E-05	0.0135782	yes
Cxcl5	WT-IGA	miR106b-93-25-/ IGA	2.10739	5.00E-05	0.0135782	yes
Lcn2	WT-IGA	miR106b-93-25-/ IGA	2.06349	5.00E-05	0.0135782	yes
Cebpb	WT-IGA	miR106b-93-25-/ IGA	2.03939	5.00E-05	0.0135782	yes
S100a9	WT-IGA	miR106b-93-25-/ IGA	2.0372	5.00E-05	0.0135782	yes
Sox10	WT-IGA	miR106b-93-25-/ IGA	2.02104	5.00E-05	0.0135782	yes
Vaultrc5	WT-IGA	miR106b-93-25-/ IGA	2.00683	5.00E-05	0.0135782	yes
Slco2a1	WT-IGA	miR106b-93-25-/ IGA	1.99534	5.00E-05	0.0135782	yes
Nfkbiz	WT-IGA	miR106b-93-25-/ IGA	1.97145	5.00E-05	0.0135782	yes
Arg1	WT-IGA	miR106b-93-25-/ IGA	1.96929	5.00E-05	0.0135782	yes
Nlrp3	WT-IGA	miR106b-93-25-/ IGA	1.9429	5.00E-05	0.0135782	yes
Gldn	WT-IGA	miR106b-93-25-/ IGA	1.93534	5.00E-05	0.0135782	yes
Serpina3m	WT-IGA	miR106b-93-25-/ IGA	1.89594	0.00015	0.0342817	yes
Clec4e	WT-IGA	miR106b-93-25-/ IGA	1.89139	5.00E-05	0.0135782	yes
Kif1a	WT-IGA	miR106b-93-25-/ IGA	1.82699	5.00E-05	0.0135782	yes
Cd163	WT-IGA	miR106b-93-25-/ IGA	1.81543	0.0001	0.0248204	yes
Egr1	WT-IGA	miR106b-93-25-/ IGA	1.81465	5.00E-05	0.0135782	yes
Syt4	WT-IGA	miR106b-93-25-/ IGA	1.79221	0.0001	0.0248204	yes
Ptx3	WT-IGA	miR106b-93-25-/ IGA	1.75132	5.00E-05	0.0135782	yes
Ccdc88b	WT-IGA	miR106b-93-25-/ IGA	1.73513	5.00E-05	0.0135782	yes

Ngfr	WT-IGA	miR106b-93-25-/- IGA	1.72648	5.00E-05	0.0135782	yes
Hbb-b1	WT-IGA	miR106b-93-25-/- IGA	1.72583	5.00E-05	0.0135782	yes
Junb	WT-IGA	miR106b-93-25-/- IGA	1.69778	5.00E-05	0.0135782	yes
Ier3	WT-IGA	miR106b-93-25-/- IGA	1.64264	5.00E-05	0.0135782	yes
Ccl8	WT-IGA	miR106b-93-25-/- IGA	1.64202	5.00E-05	0.0135782	yes
Notch4	WT-IGA	miR106b-93-25-/- IGA	1.63231	5.00E-05	0.0135782	yes
Hba-a1	WT-IGA	miR106b-93-25-/- IGA	1.63197	5.00E-05	0.0135782	yes
Ppp1r3b	WT-IGA	miR106b-93-25-/- IGA	1.6144	5.00E-05	0.0135782	yes
Ccl12	WT-IGA	miR106b-93-25-/- IGA	1.5836	0.00015	0.0342817	yes
Apold1	WT-IGA	miR106b-93-25-/- IGA	1.57845	5.00E-05	0.0135782	yes
Cxcr2	WT-IGA	miR106b-93-25-/- IGA	1.57405	5.00E-05	0.0135782	yes
Itgb3	WT-IGA	miR106b-93-25-/- IGA	1.56113	5.00E-05	0.0135782	yes
Nova2	WT-IGA	miR106b-93-25-/- IGA	1.55036	5.00E-05	0.0135782	yes
Zfp36	WT-IGA	miR106b-93-25-/- IGA	1.53676	5.00E-05	0.0135782	yes
Fos	WT-IGA	miR106b-93-25-/- IGA	1.53385	5.00E-05	0.0135782	yes
Cchcr1	WT-IGA	miR106b-93-25-/- IGA	1.48943	5.00E-05	0.0135782	yes
Hlx	WT-IGA	miR106b-93-25-/- IGA	1.48696	5.00E-05	0.0135782	yes
Beta-s	WT-IGA	miR106b-93-25-/- IGA	1.45452	5.00E-05	0.0135782	yes
Cd14	WT-IGA	miR106b-93-25-/- IGA	1.45164	5.00E-05	0.0135782	yes
Adamts15	WT-IGA	miR106b-93-25-/- IGA	1.40501	5.00E-05	0.0135782	yes
Adamts4	WT-IGA	miR106b-93-25-/- IGA	1.39261	0.0002	0.0423541	yes
Adamts1	WT-IGA	miR106b-93-25-/- IGA	1.38386	5.00E-05	0.0135782	yes
Hba-a1	WT-IGA	miR106b-93-25-/- IGA	1.37775	5.00E-05	0.0135782	yes
Sema6b	WT-IGA	miR106b-93-25-/- IGA	1.35056	5.00E-05	0.0135782	yes
Zfp703	WT-IGA	miR106b-93-25-/- IGA	1.32306	0.0001	0.0248204	yes
Slc2a1	WT-IGA	miR106b-93-25-/- IGA	1.25745	0.00015	0.0342817	yes
Ier5	WT-IGA	miR106b-93-25-/- IGA	1.21031	0.00015	0.0342817	yes

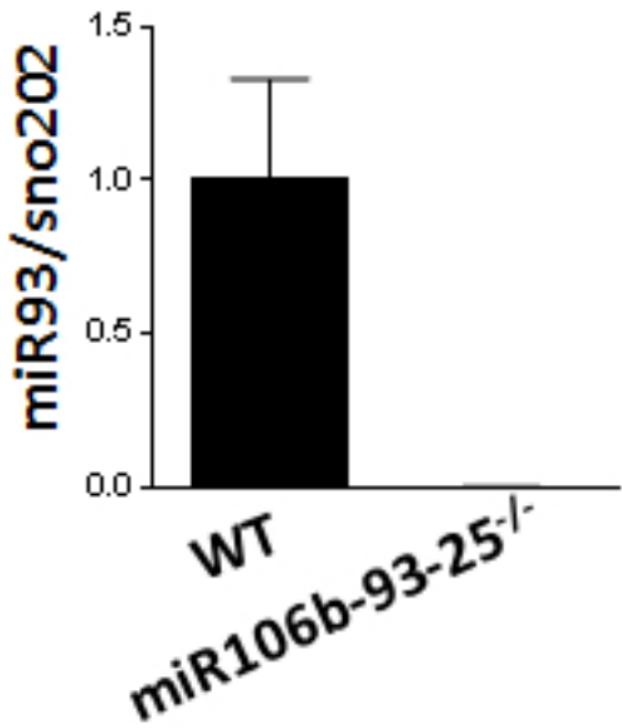
Table-1: List of upregulated genes in miR106b-93-25-/- ischemic-muscle compared to WT ischemic muscle from RNA-Seq analysis. n=5. q<0.05 considered significant. Differentially expressed genes (at a false discovery rate (FDR) of < 0.05) were identified using Cufflinks Assembly and Differential Expression v1.1.0 application.

Table-2 RNA-Seq analysis of down regulated genes in miR106b-93-25-/- ischemic-muscle vs. WT ischemic muscle

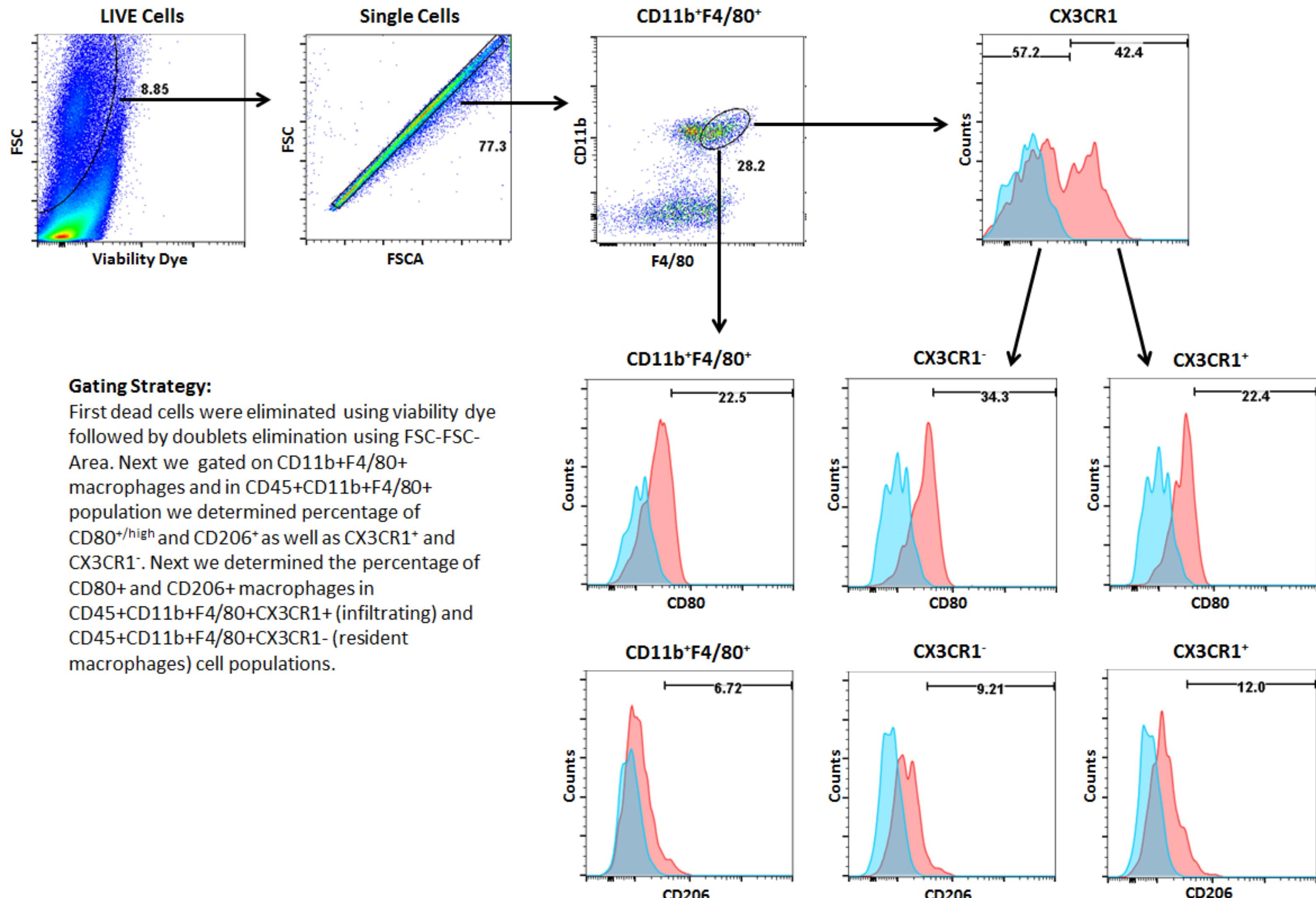
gene	sample_1	sample_2	log2(fold_change)	p_value	q_value	significant
C1qtnf3	WT-IGA	miR106b-93-25-/- IGA	-3.40205	5.00E-05	0.0135782	yes
Myh3	WT-IGA	miR106b-93-25-/- IGA	-3.37347	5.00E-05	0.0135782	yes
Actc1	WT-IGA	miR106b-93-25-/- IGA	-2.87725	5.00E-05	0.0135782	yes
Myh8	WT-IGA	miR106b-93-25-/- IGA	-2.69855	5.00E-05	0.0135782	yes
Mfap4	WT-IGA	miR106b-93-25-/- IGA	-2.31477	5.00E-05	0.0135782	yes
Myog	WT-IGA	miR106b-93-25-/- IGA	-2.22416	5.00E-05	0.0135782	yes
Myl4	WT-IGA	miR106b-93-25-/- IGA	-1.95579	0.0002	0.0423541	yes
Postn	WT-IGA	miR106b-93-25-/- IGA	-1.93927	0.00015	0.0342817	yes
Pnmal2	WT-IGA	miR106b-93-25-/- IGA	-1.8455	5.00E-05	0.0135782	yes
Tnmd	WT-IGA	miR106b-93-25-/- IGA	-1.83319	5.00E-05	0.0135782	yes
Igf2	WT-IGA	miR106b-93-25-/- IGA	-1.80664	0.0001	0.0248204	yes
Cthrc1	WT-IGA	miR106b-93-25-/- IGA	-1.78116	5.00E-05	0.0135782	yes
Sntb1	WT-IGA	miR106b-93-25-/- IGA	-1.6512	0.0001	0.0248204	yes
Aif1l	WT-IGA	miR106b-93-25-/- IGA	-1.59561	5.00E-05	0.0135782	yes
Itm2a	WT-IGA	miR106b-93-25-/- IGA	-1.5718	5.00E-05	0.0135782	yes
Sox11	WT-IGA	miR106b-93-25-/- IGA	-1.55397	5.00E-05	0.0135782	yes
Capn6	WT-IGA	miR106b-93-25-/- IGA	-1.5369	5.00E-05	0.0135782	yes
Megf10	WT-IGA	miR106b-93-25-/- IGA	-1.4987	0.0002	0.0423541	yes
Cilp	WT-IGA	miR106b-93-25-/- IGA	-1.48801	0.0002	0.0423541	yes
Kera	WT-IGA	miR106b-93-25-/- IGA	-1.48061	0.0002	0.0423541	yes
Csrp2	WT-IGA	miR106b-93-25-/- IGA	-1.39699	5.00E-05	0.0135782	yes
Vash2	WT-IGA	miR106b-93-25-/- IGA	-1.34814	5.00E-05	0.0135782	yes
F2r	WT-IGA	miR106b-93-25-/- IGA	-1.2617	5.00E-05	0.0135782	yes
Tubb2b	WT-IGA	miR106b-93-25-/- IGA	-1.22261	0.00015	0.0342817	yes

Table-2: List of downregulated genes in miR106b-93-25-/- ischemic muscle compared to WT ischemic muscle from RNA-Seq analysis. n=5. q<0.05 considered significant. Differentially expressed genes (at a false discovery rate (FDR) of < 0.05) were identified using Cufflinks Assembly and Differential Expression v1.1.0 application.

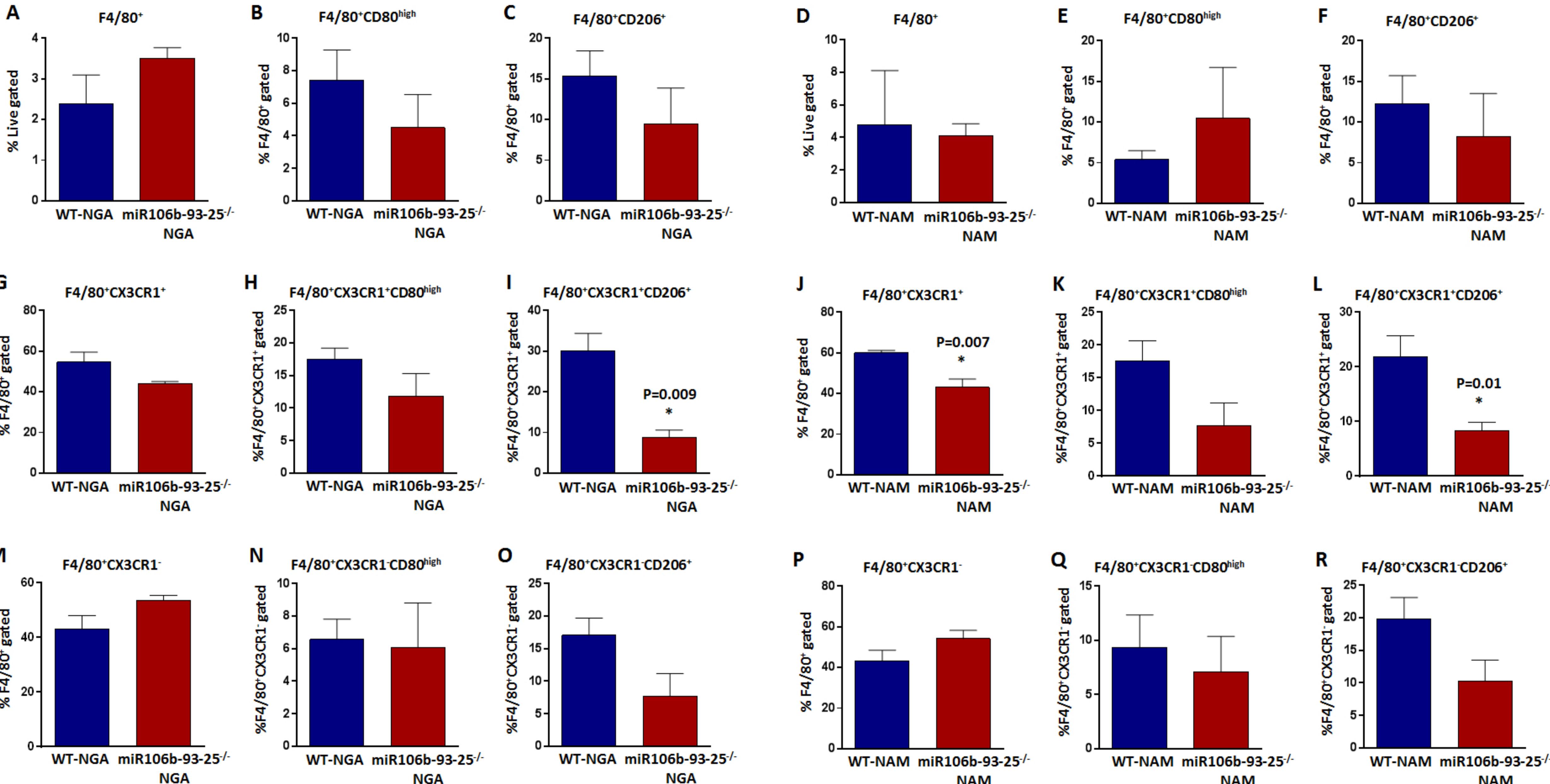
Supplemental Figure-1



Supplemental Figure-2

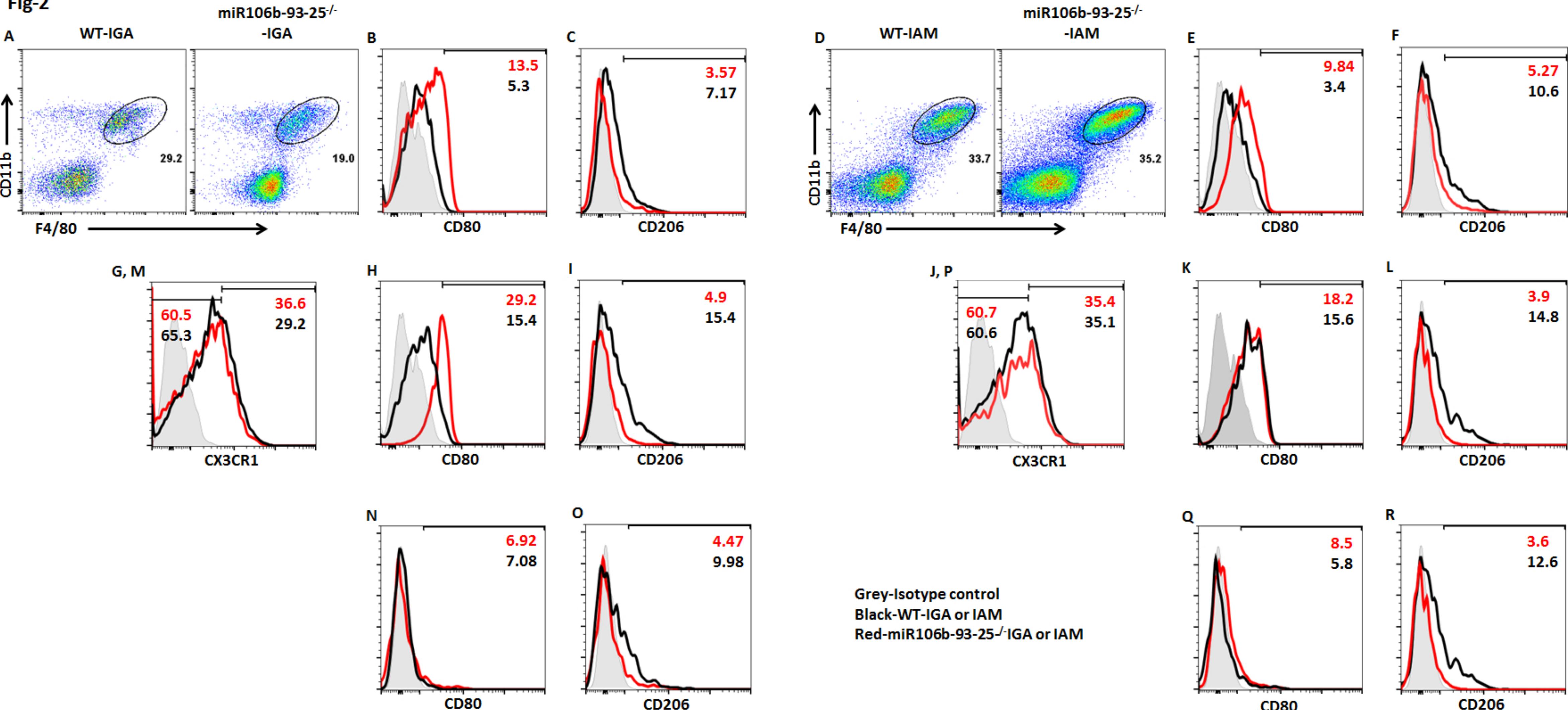


Supplemental Figure-3



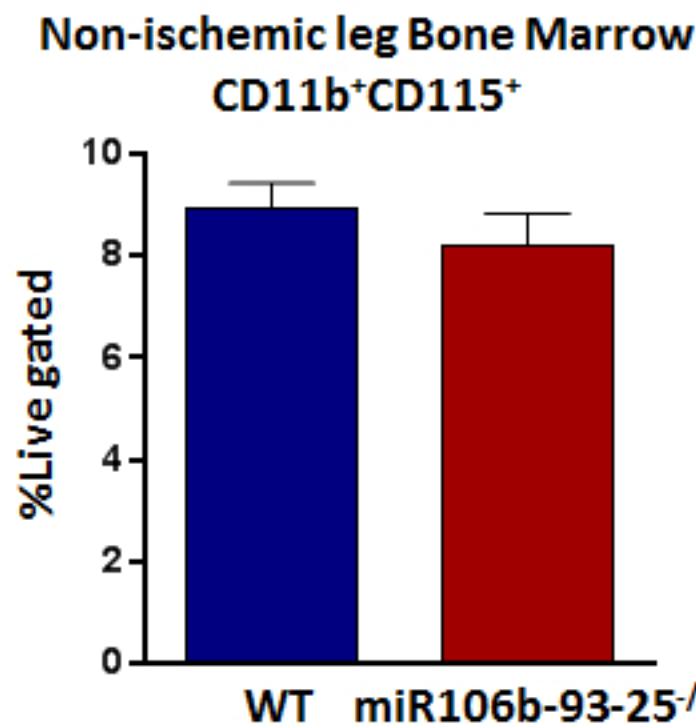
Supplemental Figure-4

Fig-2

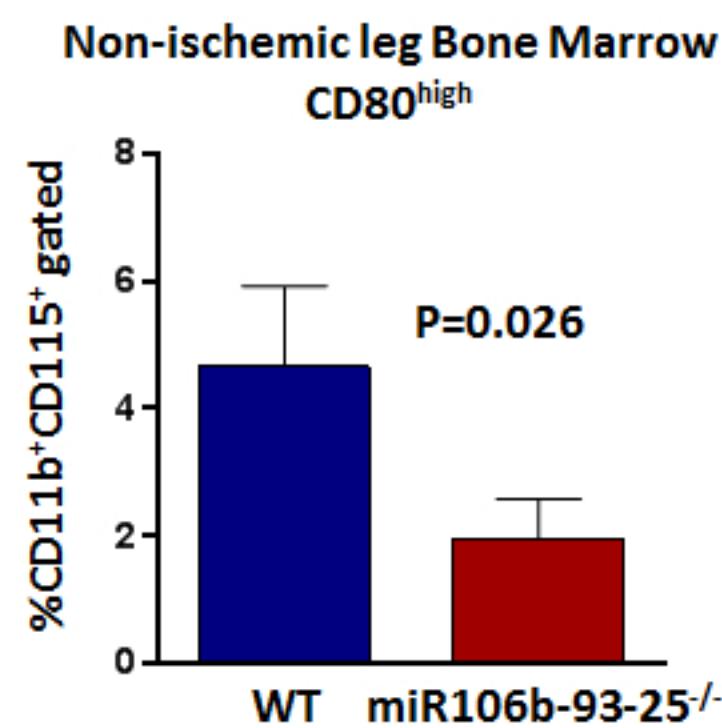


Supplemental Figure-5

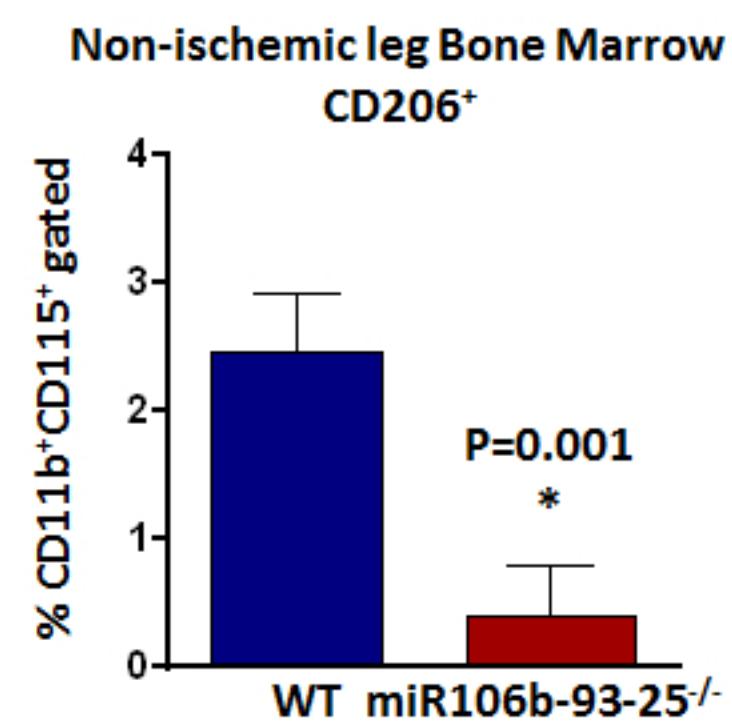
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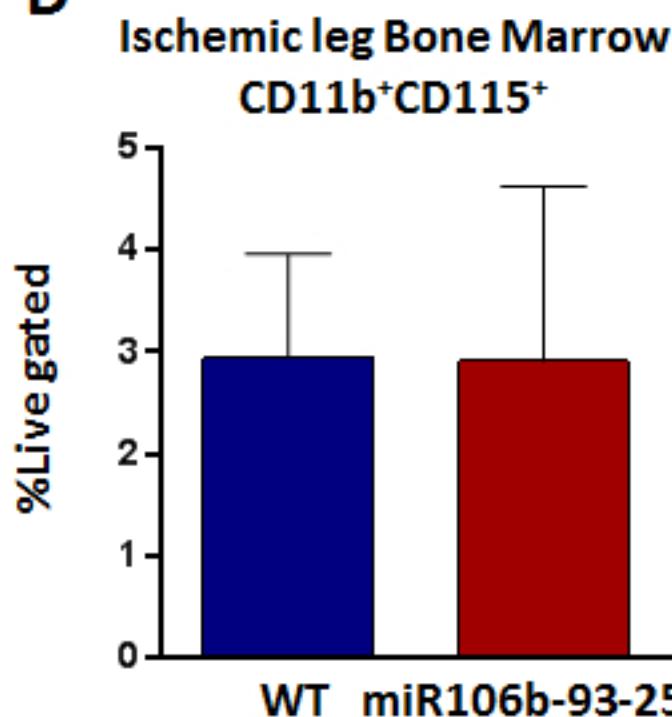
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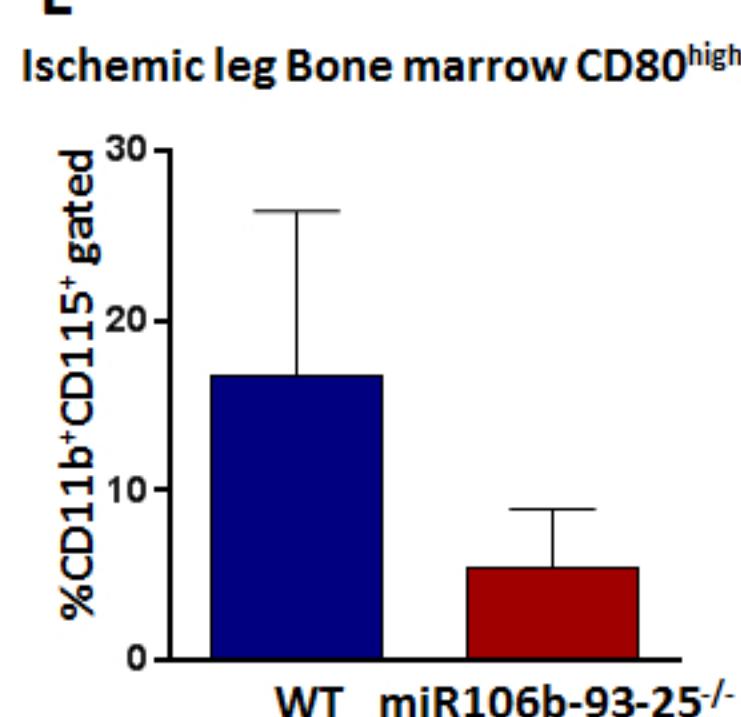
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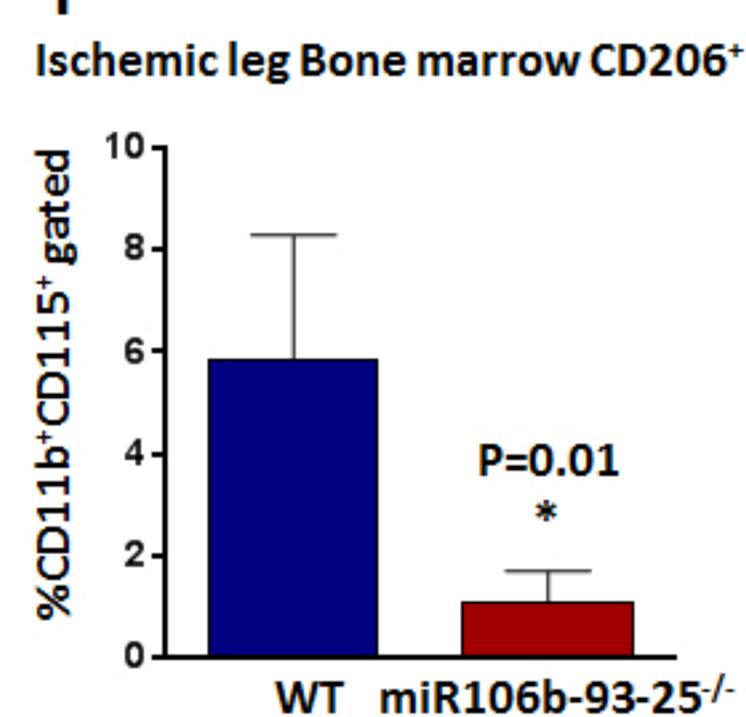
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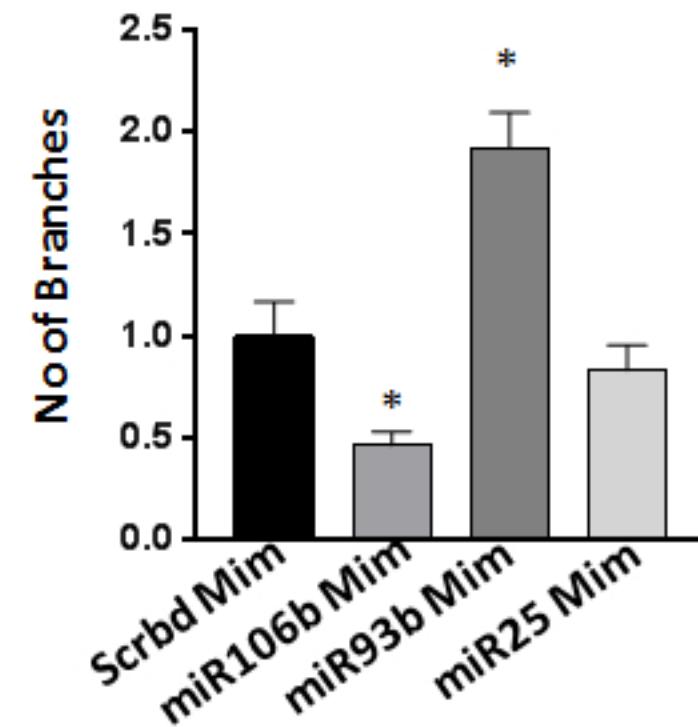
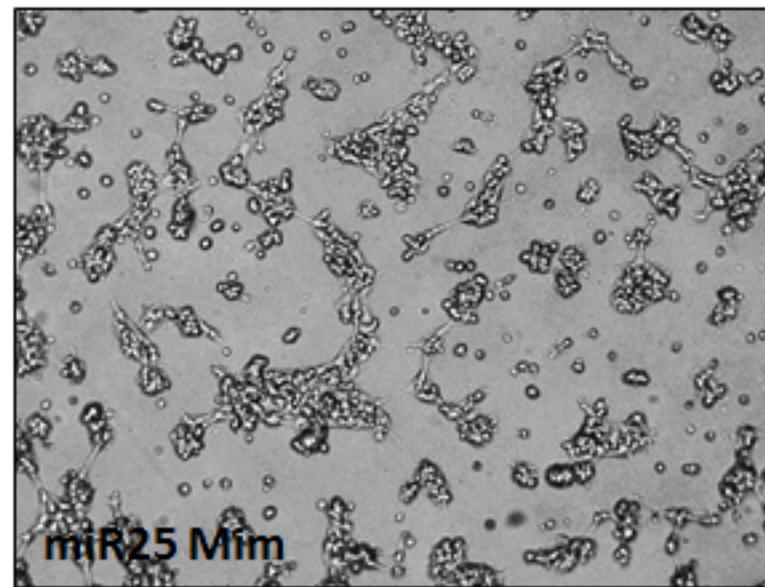
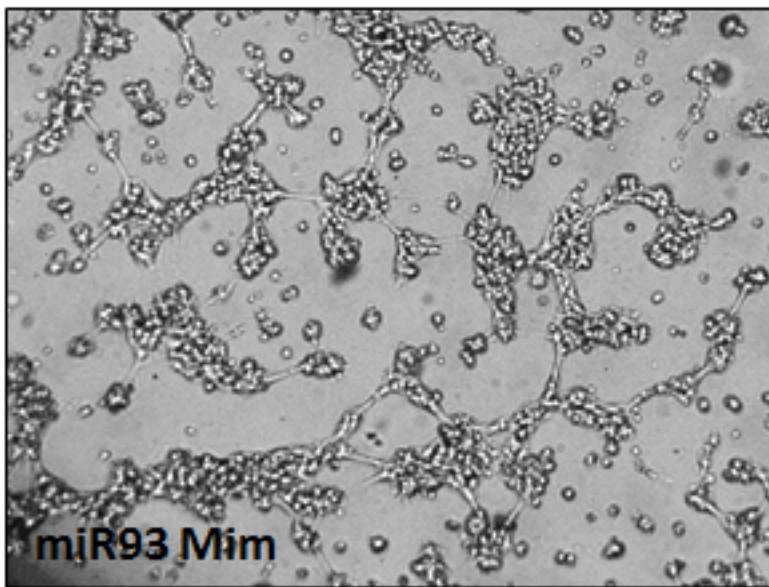
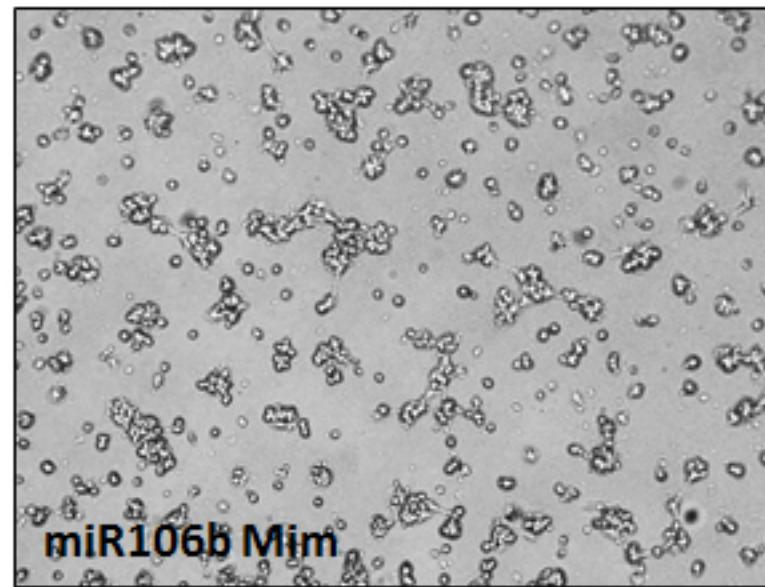
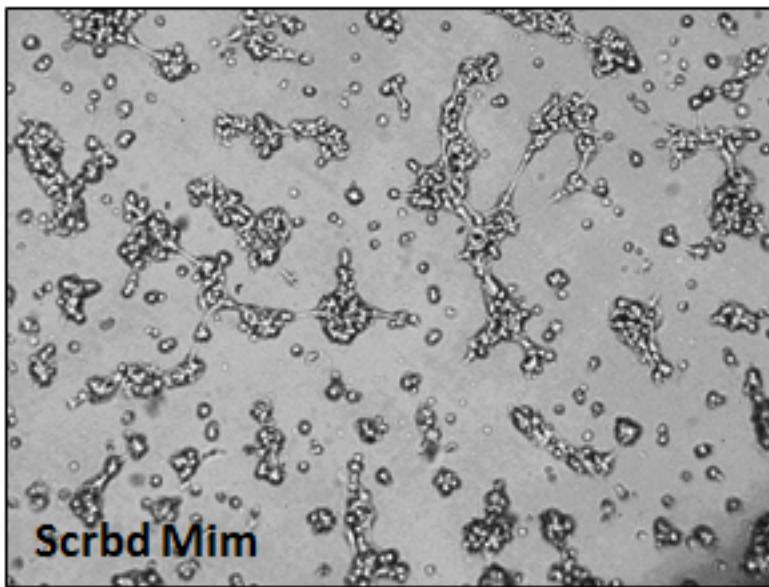
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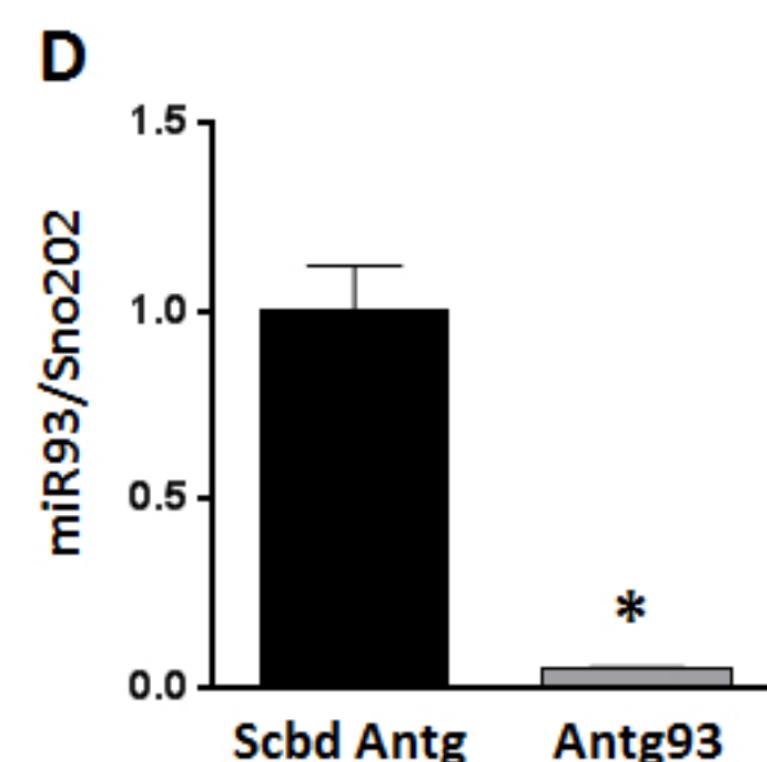
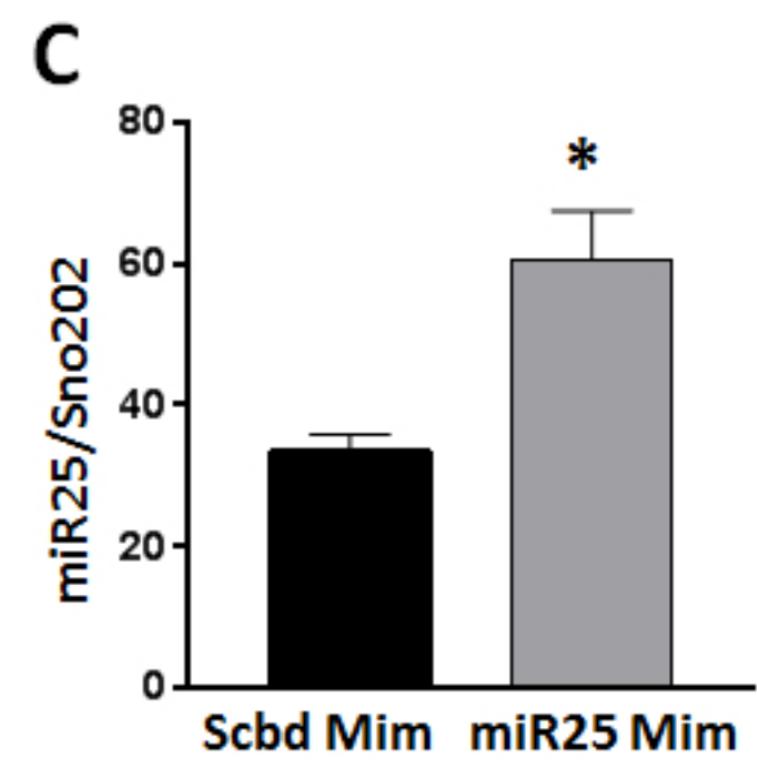
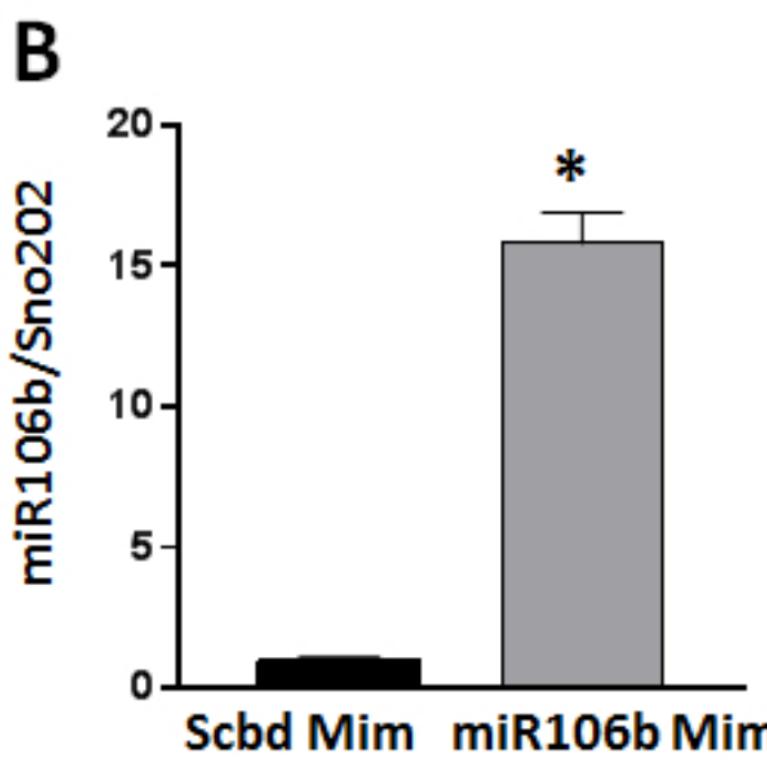
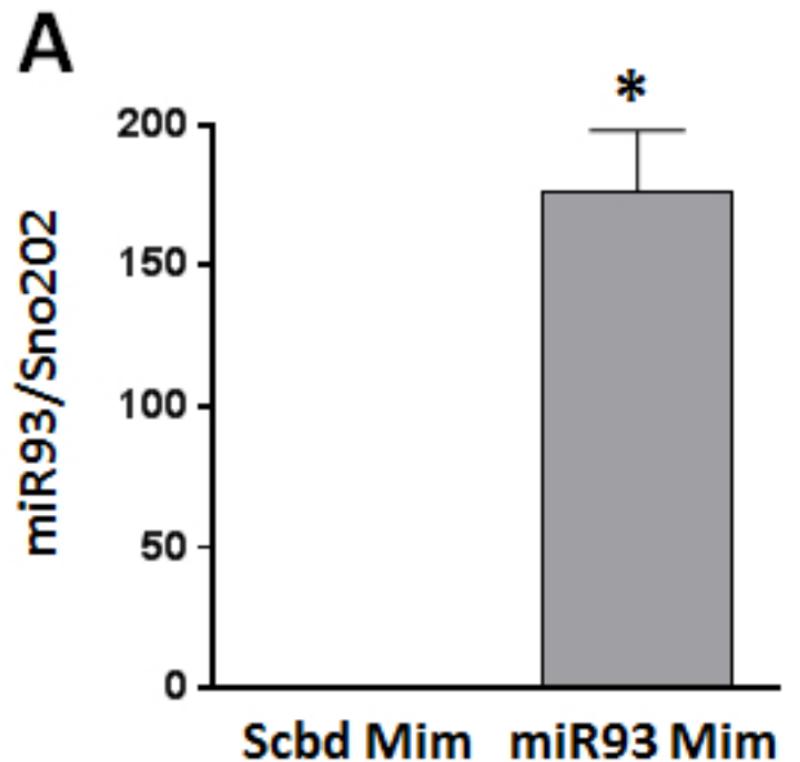
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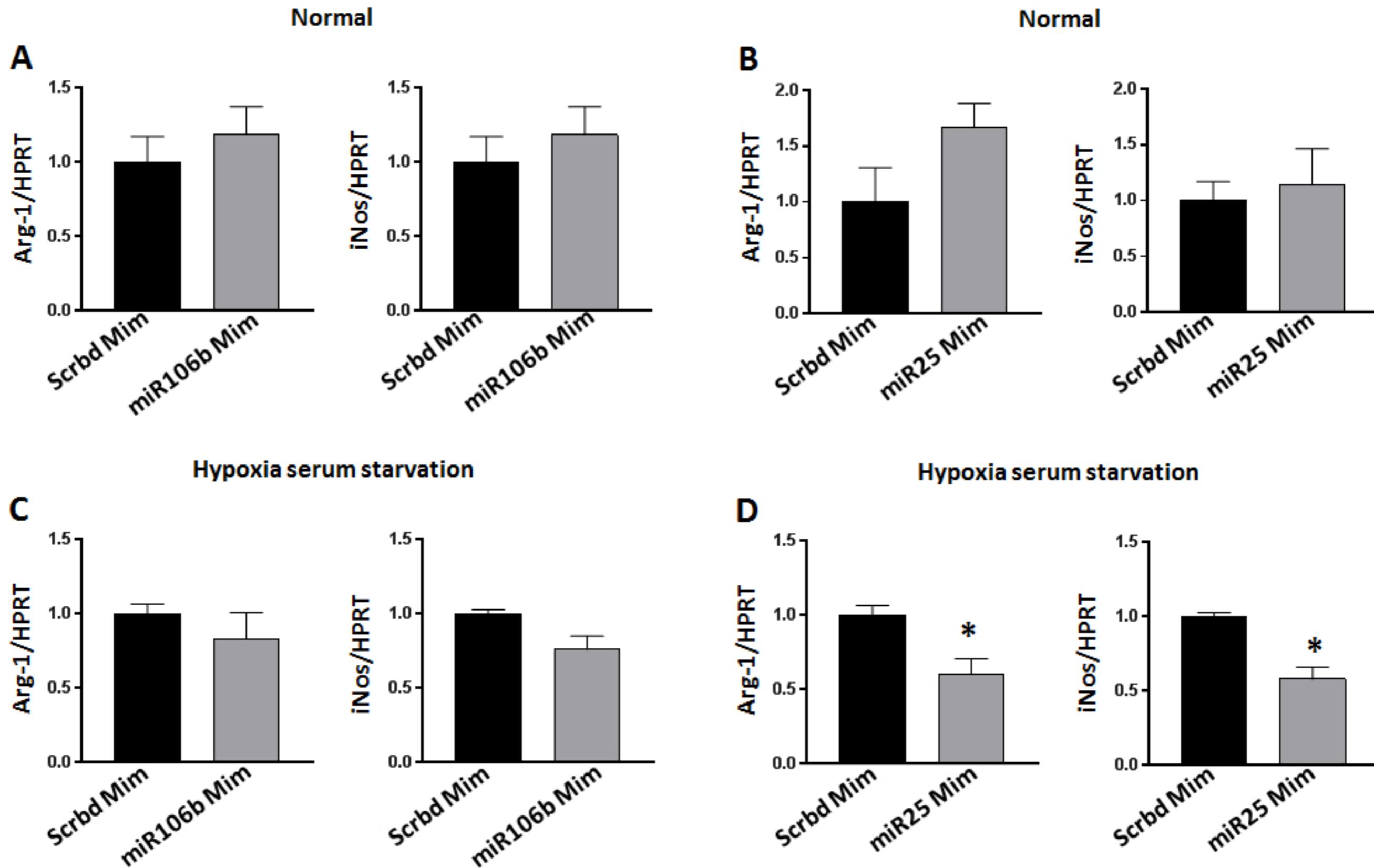
Supplemental Figure-6



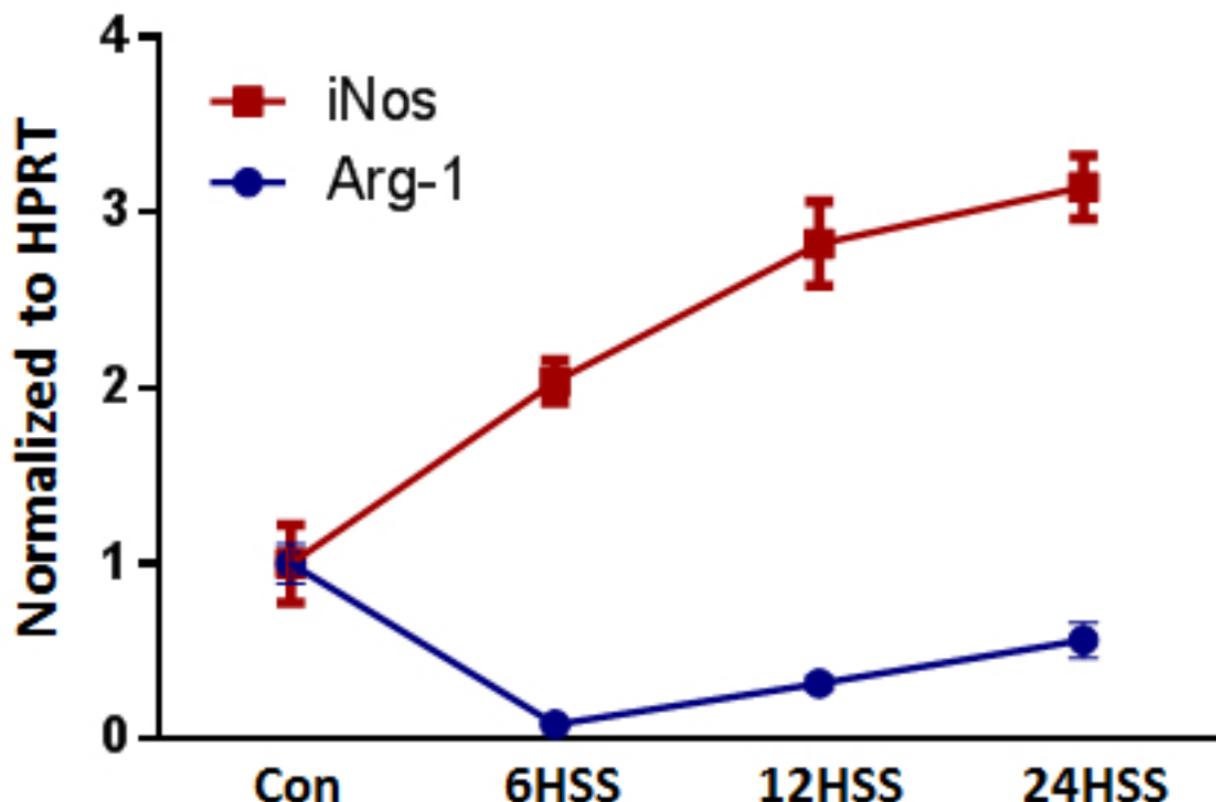
Supplemental Figure-7



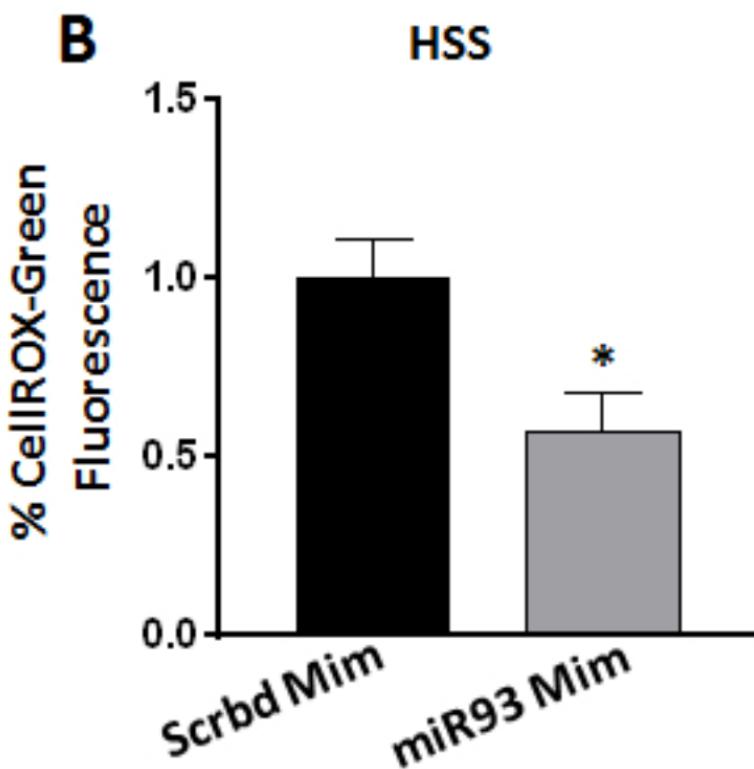
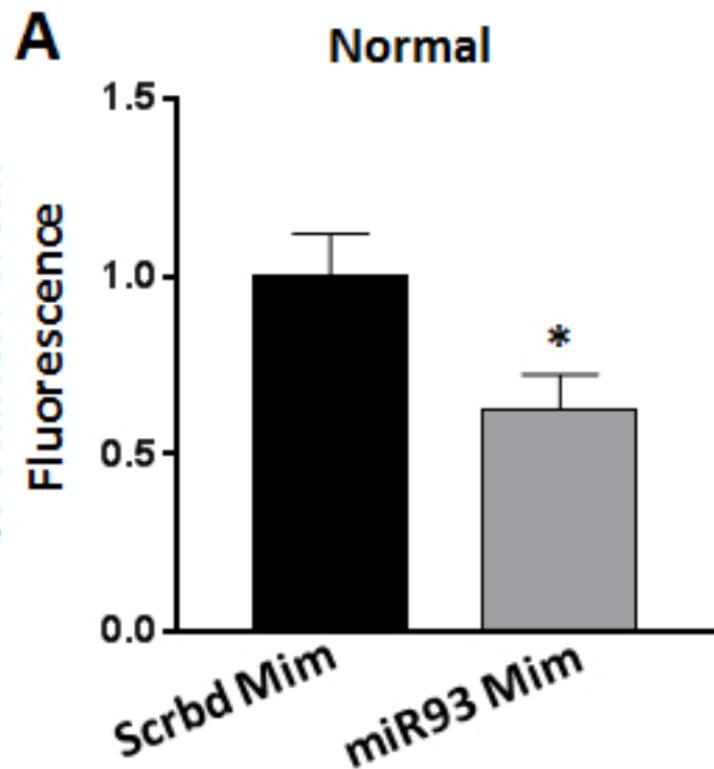
Supplemental Figure-8



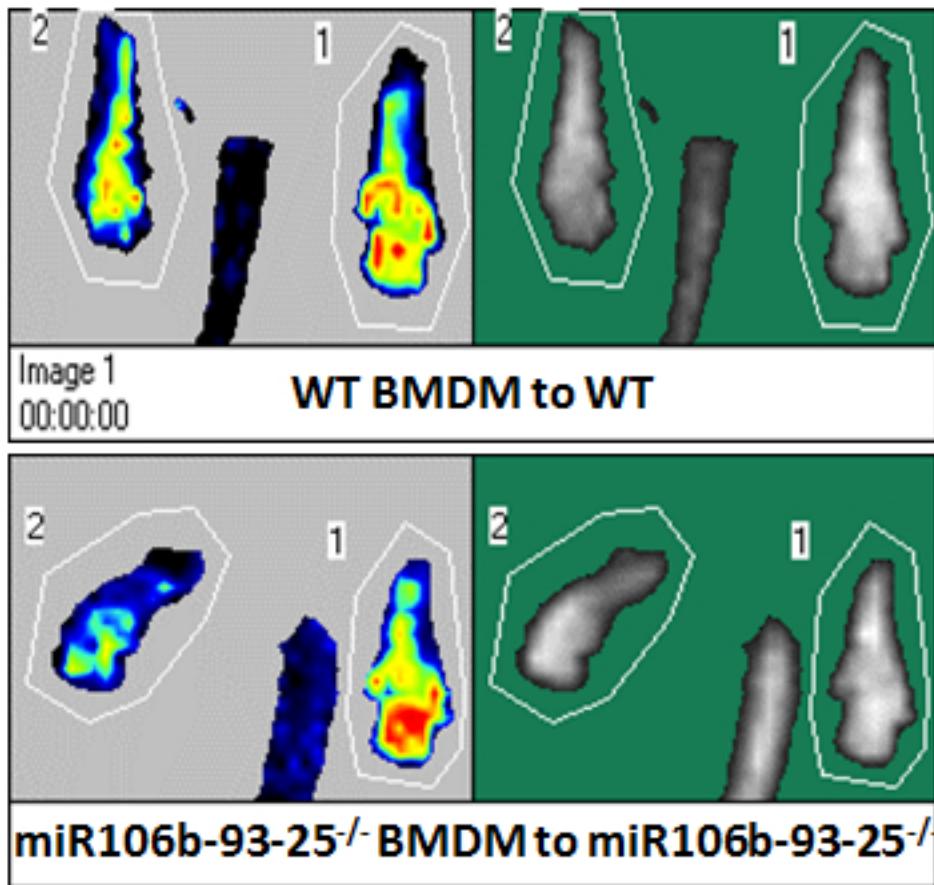
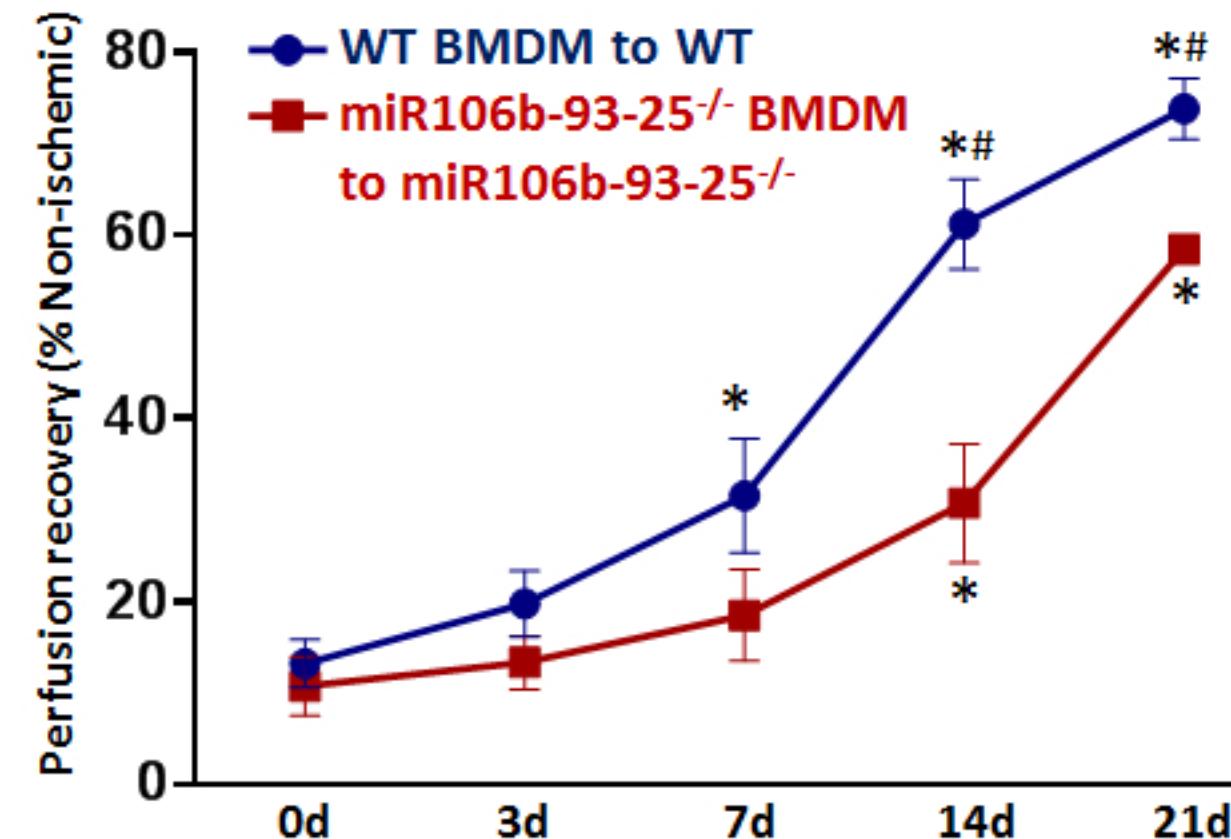
Supplemental Figure-9



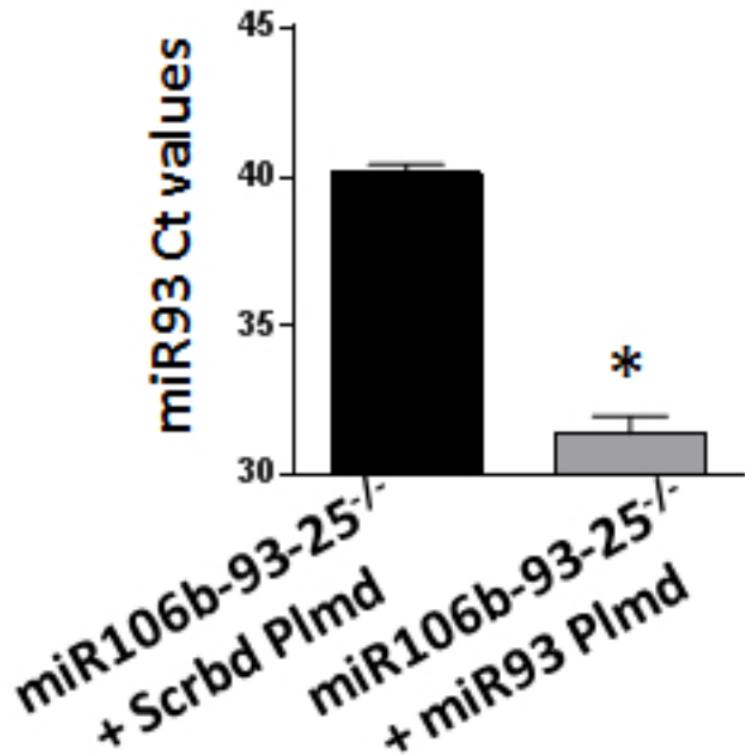
Supplemental Figure-10



Supplemental Figure-11

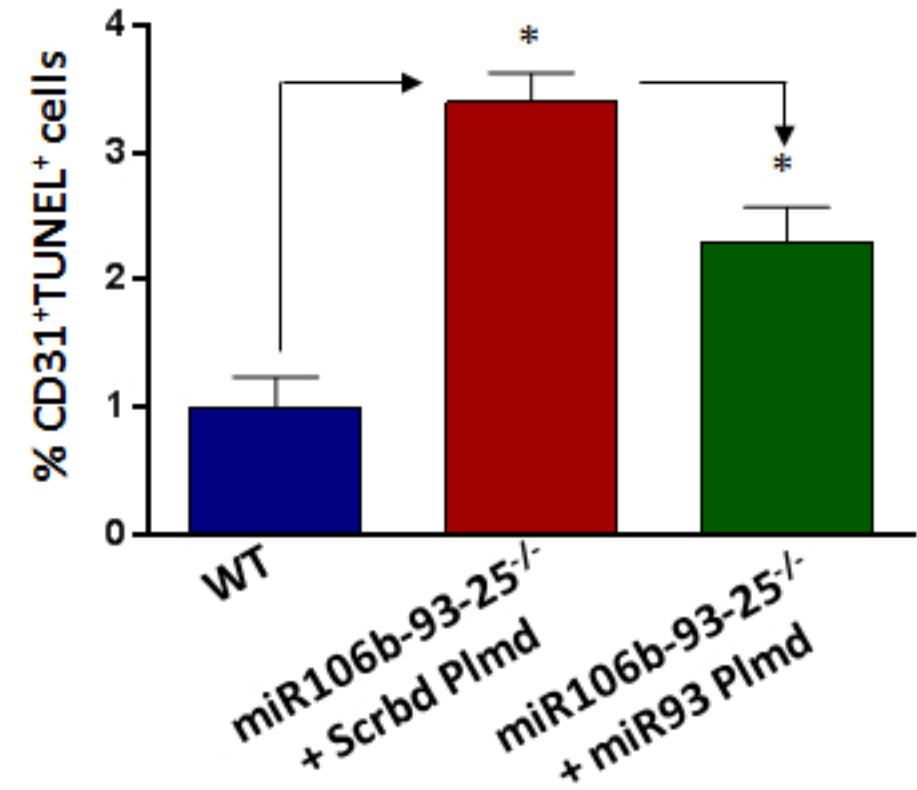
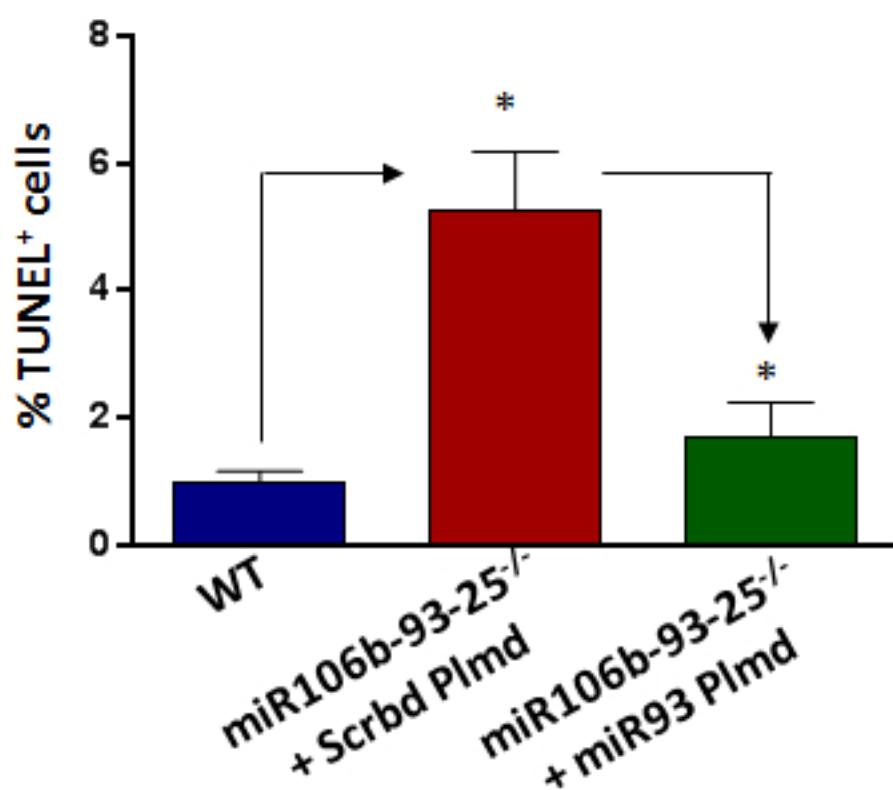
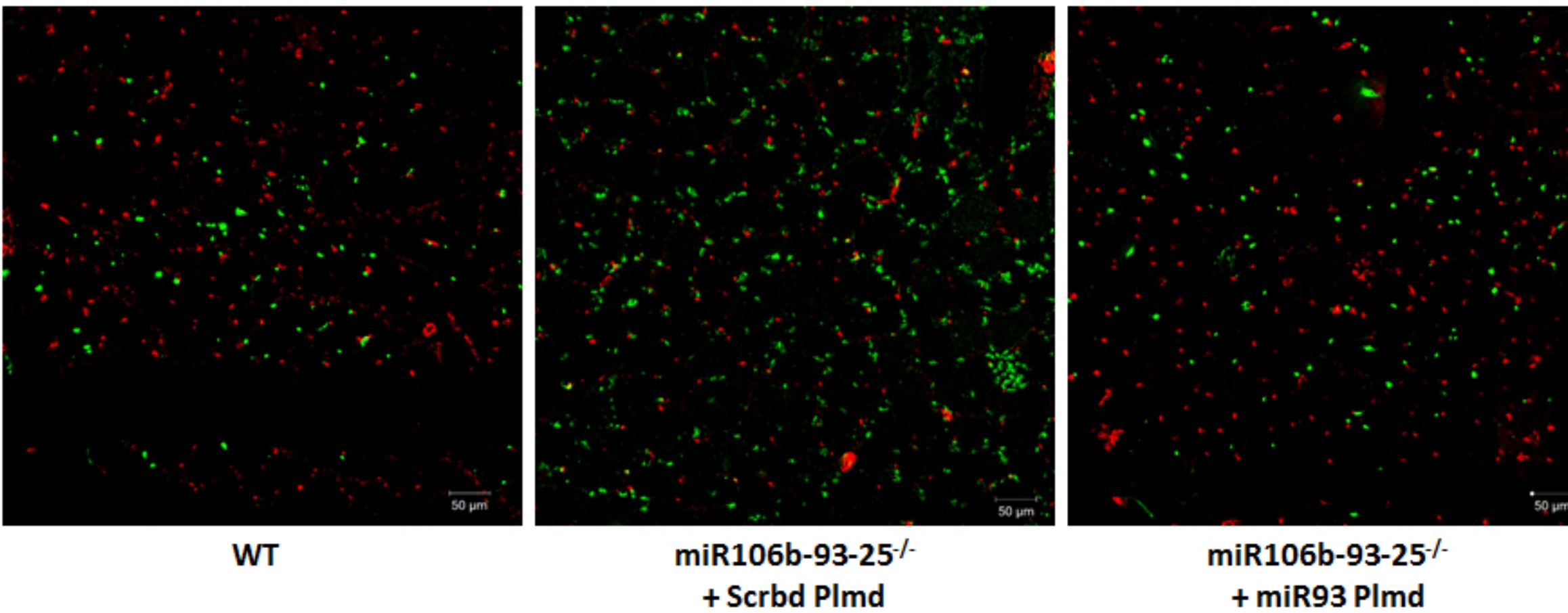


Supplemental Figure-12

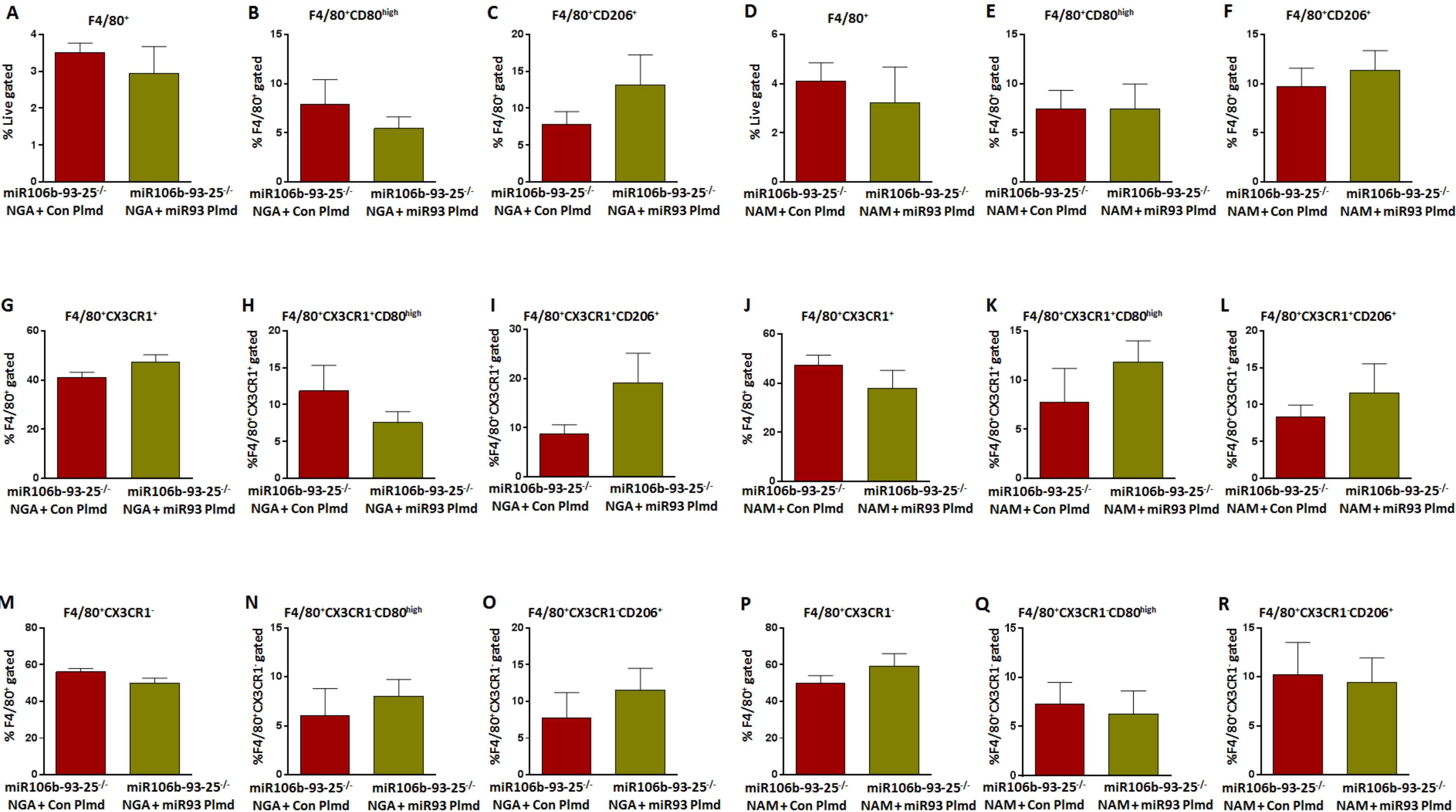


Supplemental Figure-13

Analysis of apoptotic (TUNEL+) endothelial cells in ischemic muscle

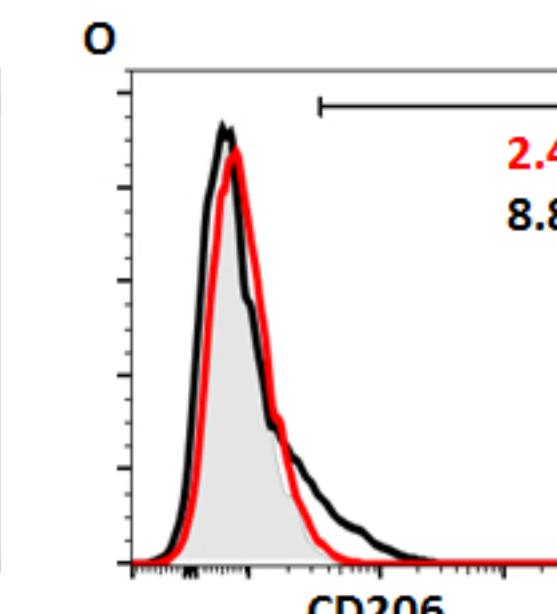
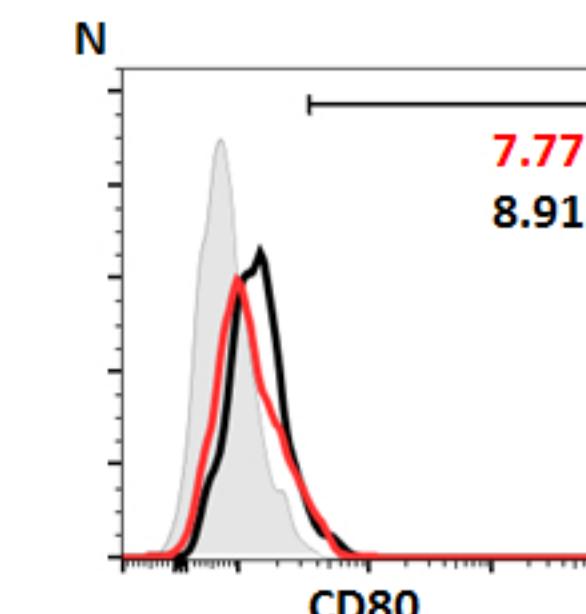
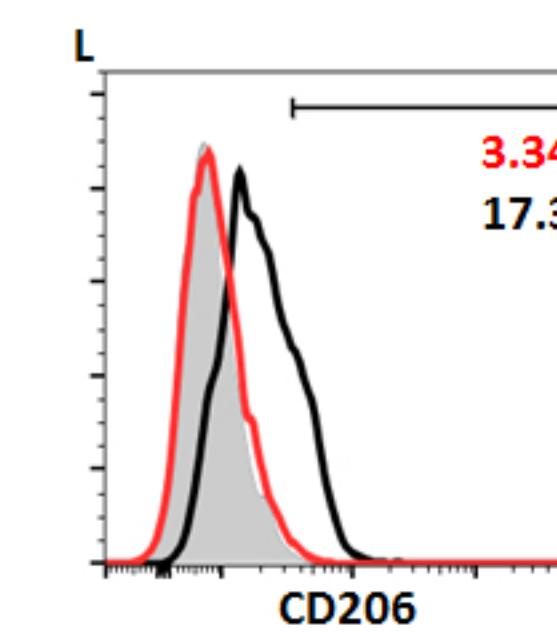
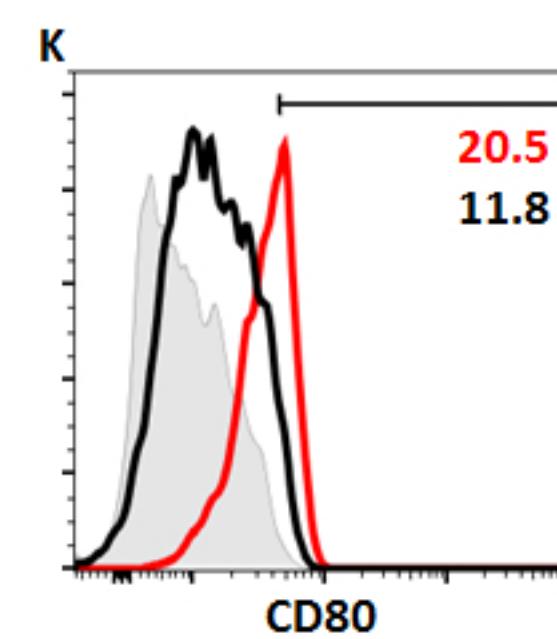
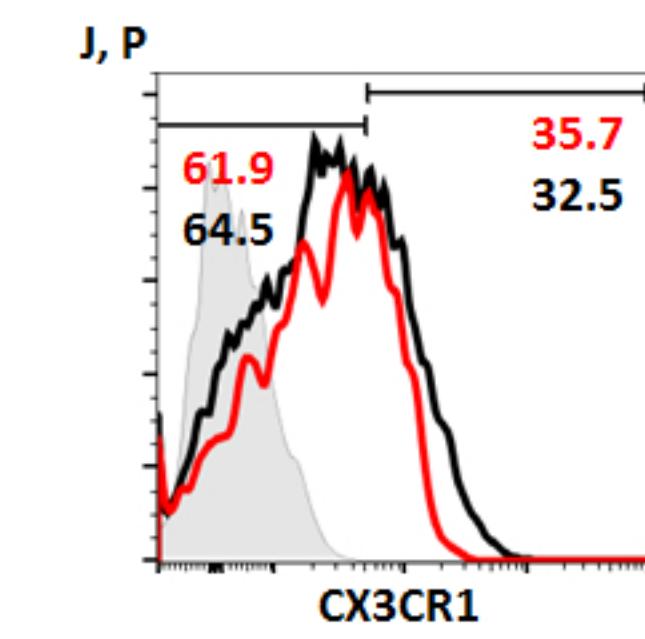
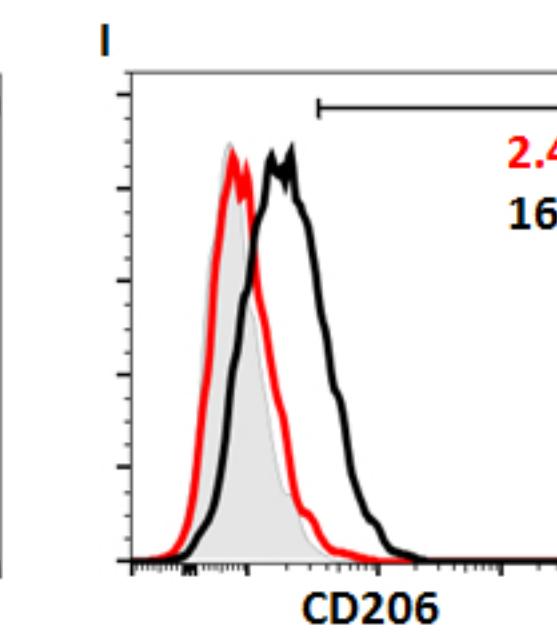
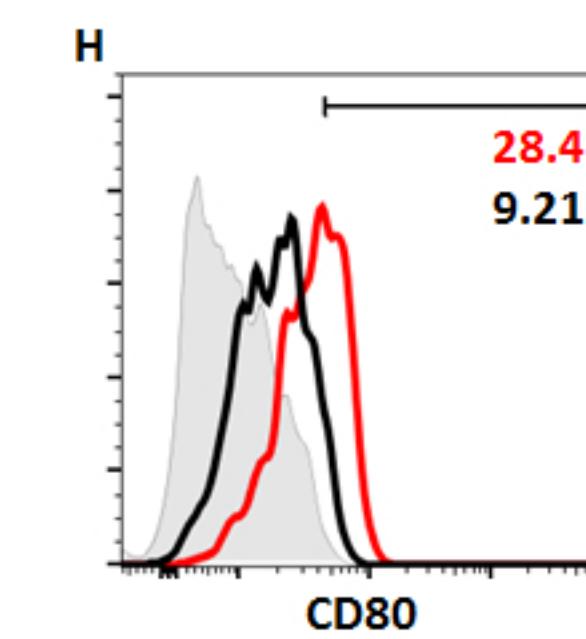
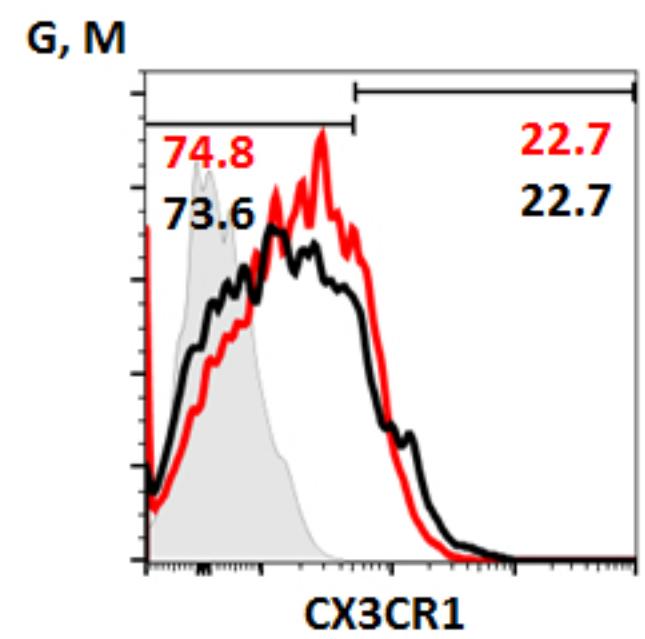
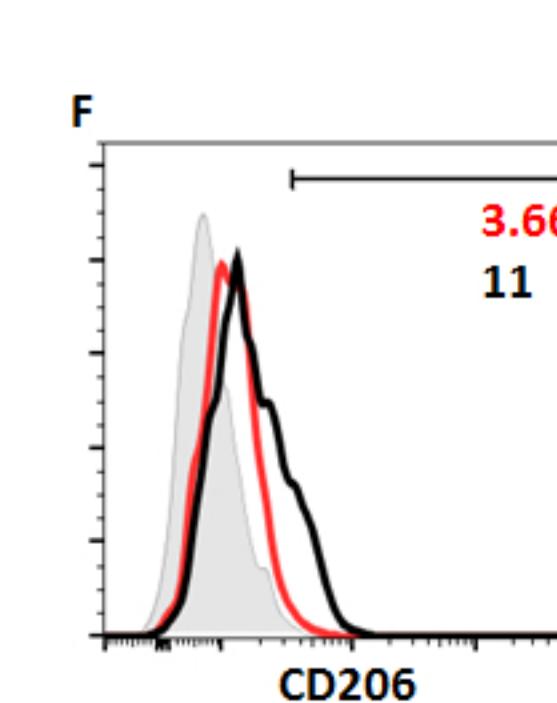
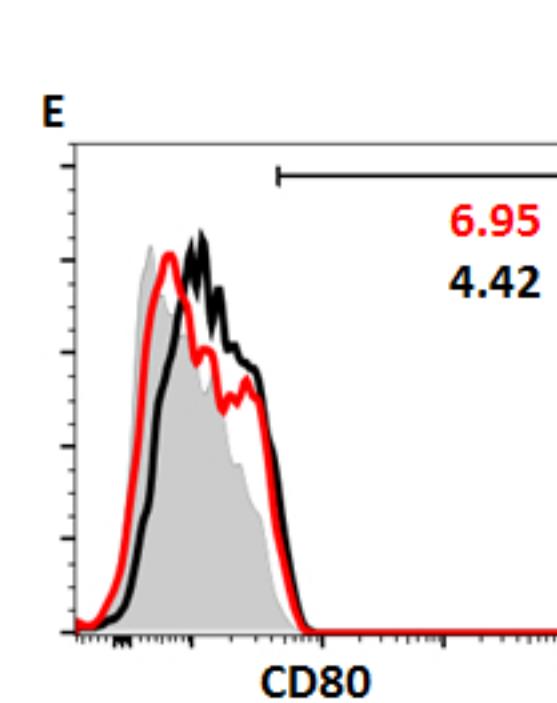
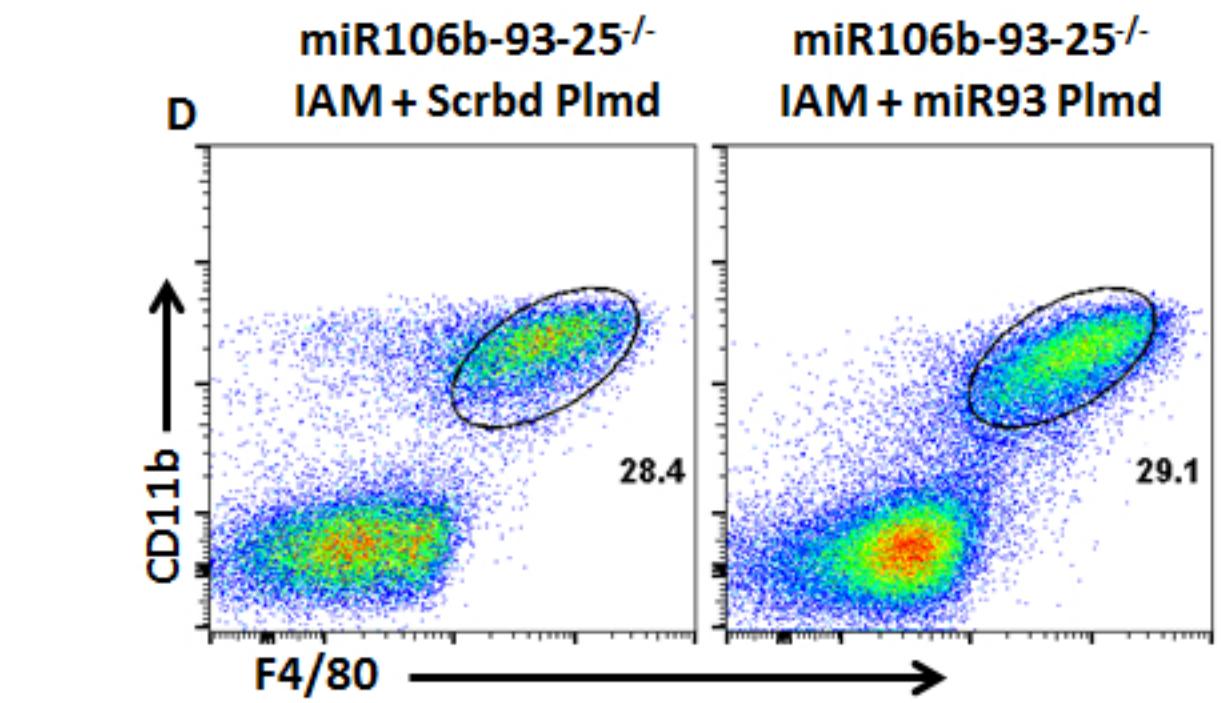
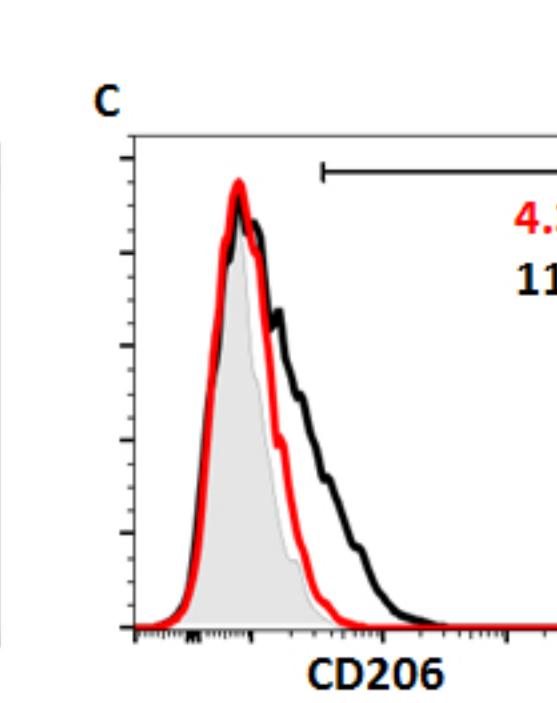
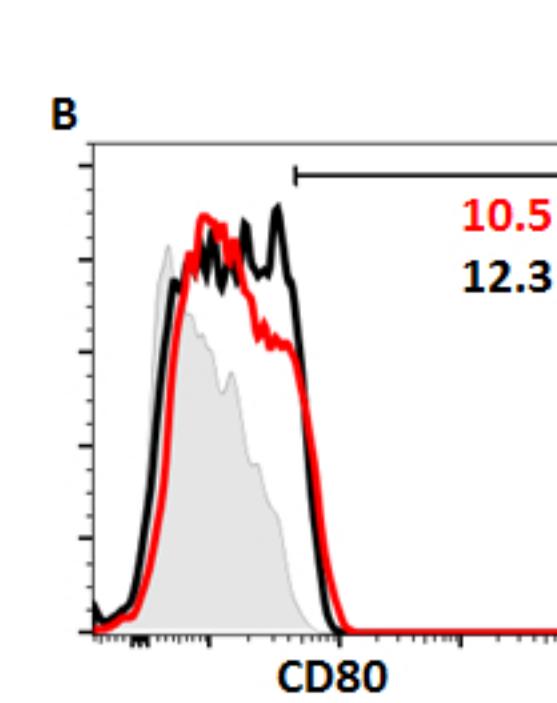
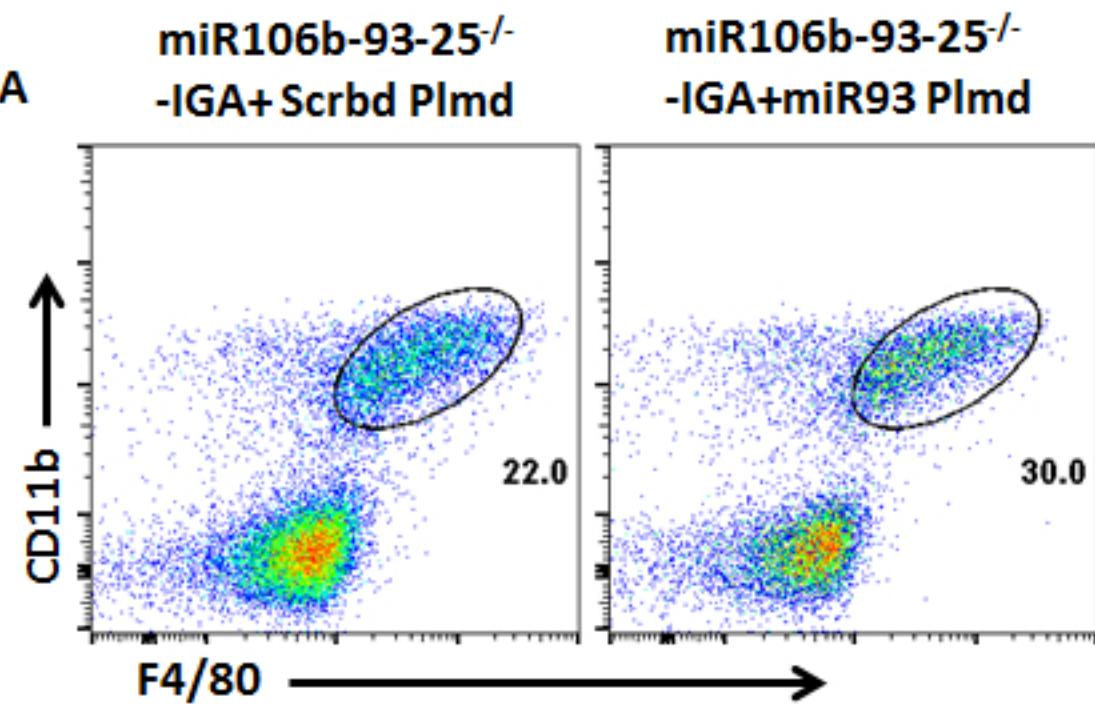


Supplemental Figure-14

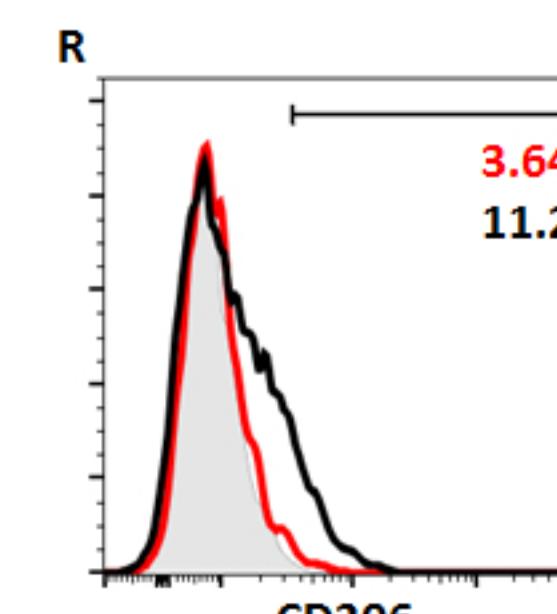
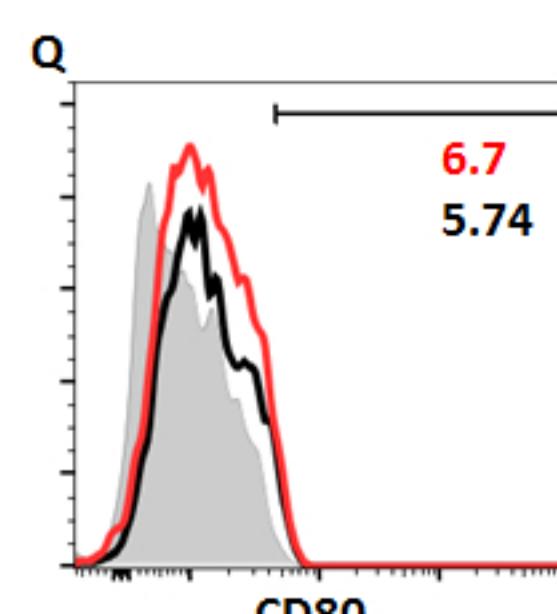


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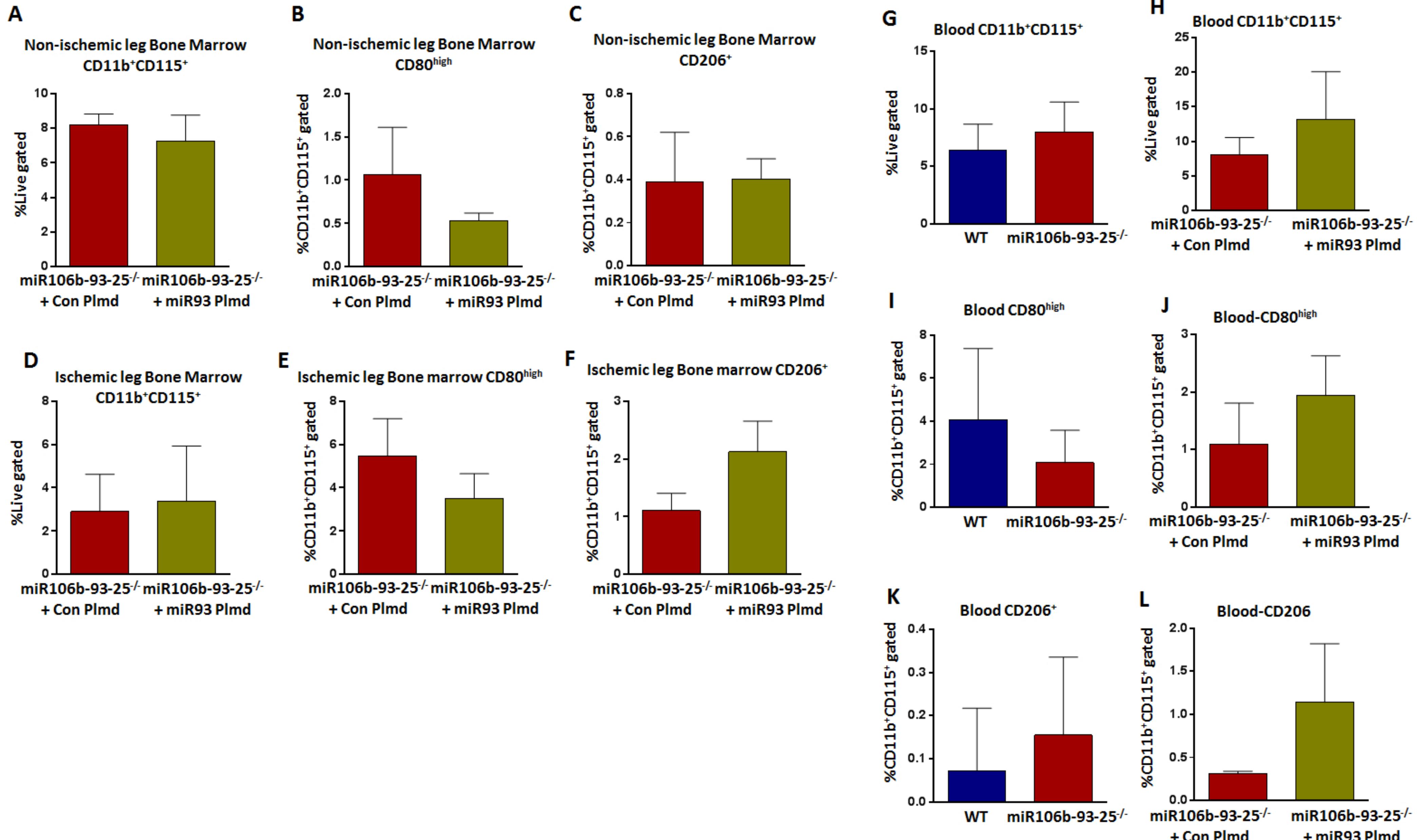
Fig-6



Grey-Isotype control
Black-miR106b-93-25^{-/-} IGA + Scrbd Plmd
Red-miR106b-93-25^{-/-} IGA + miR93 Plmd

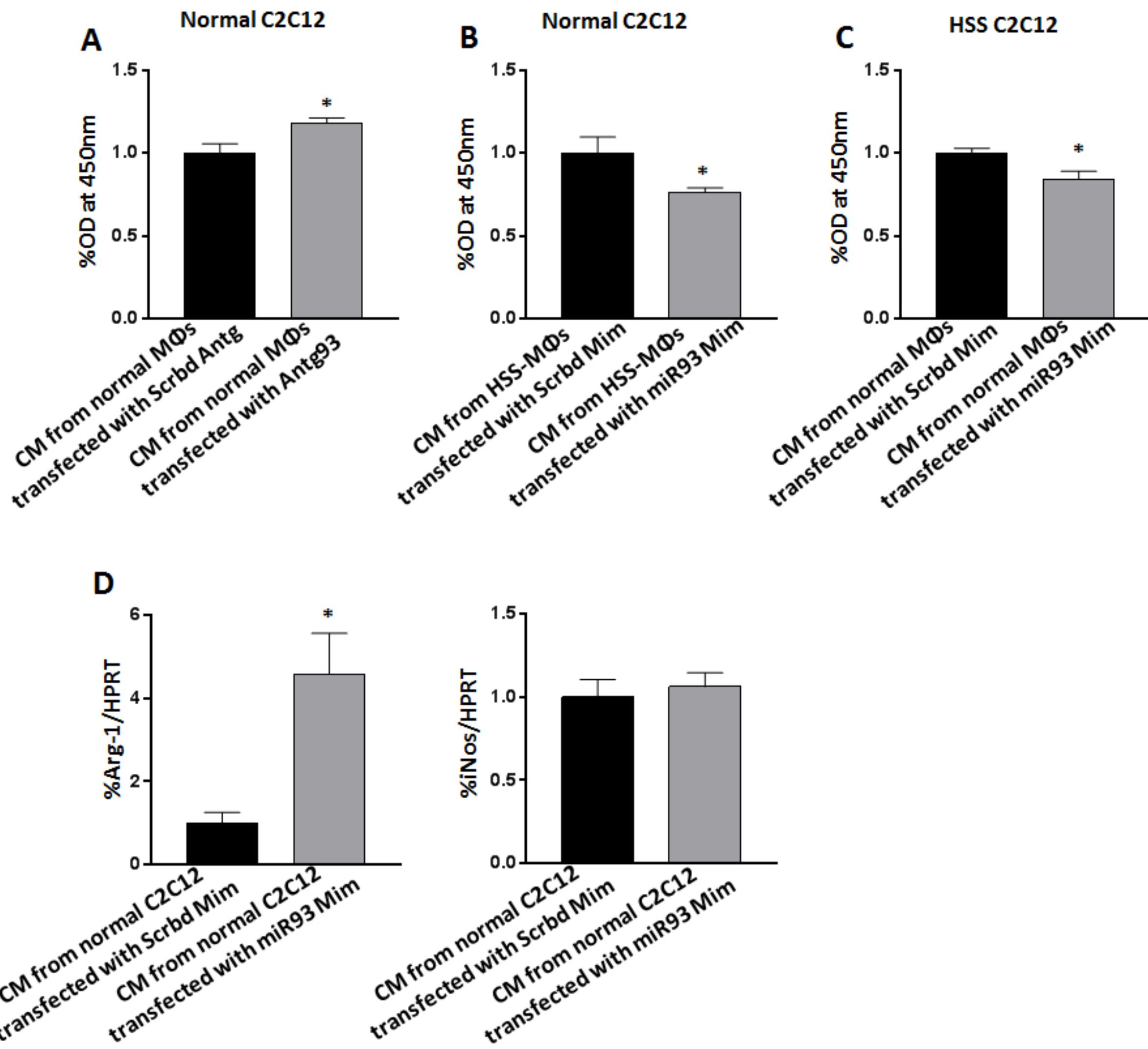


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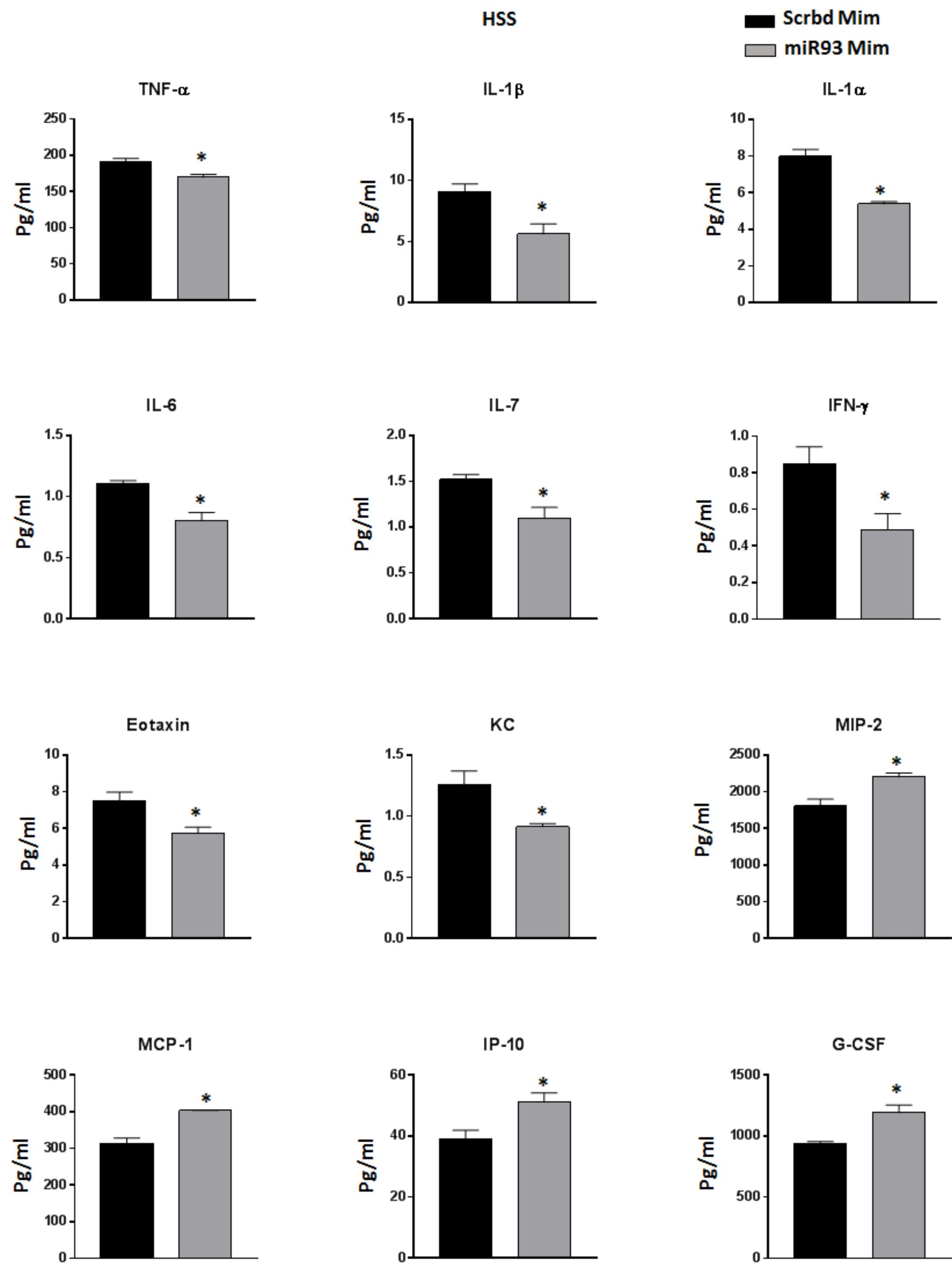


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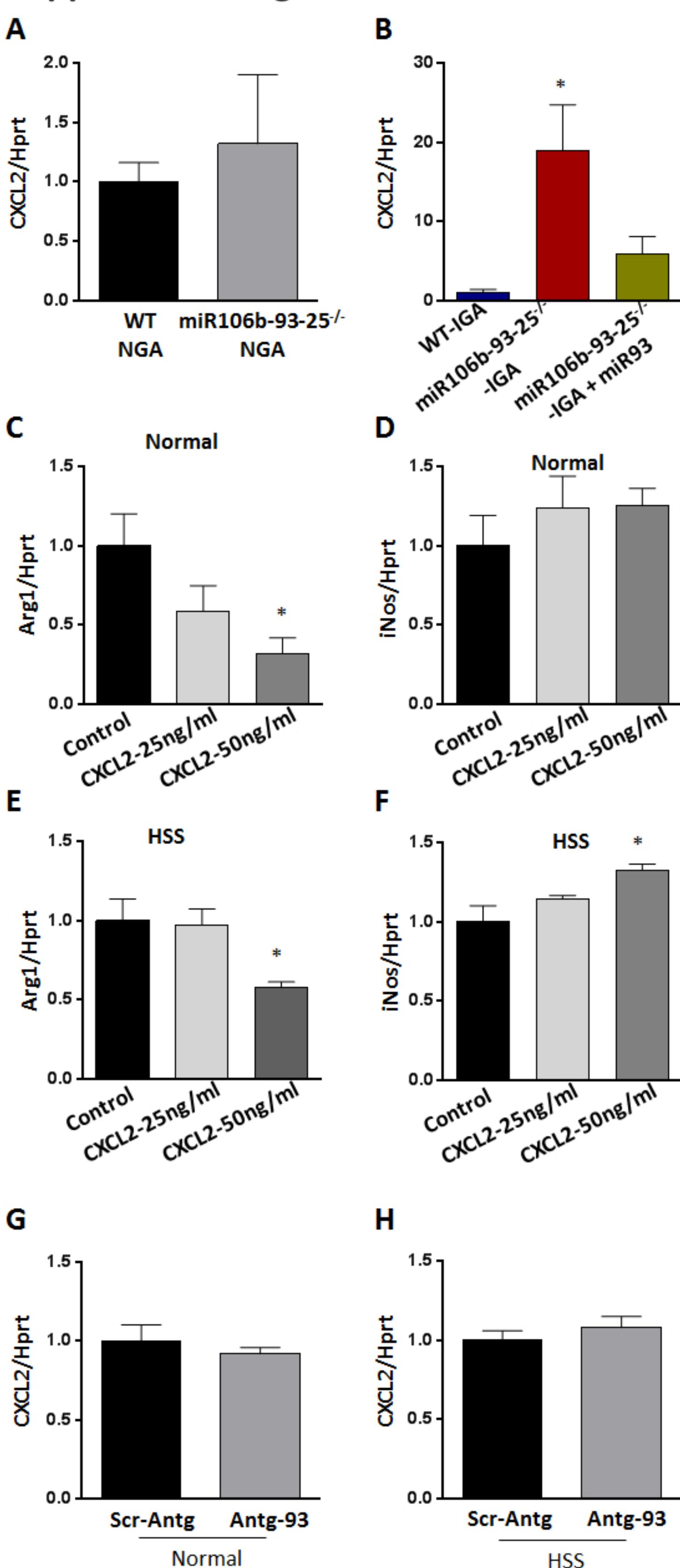
TUNEL Assay



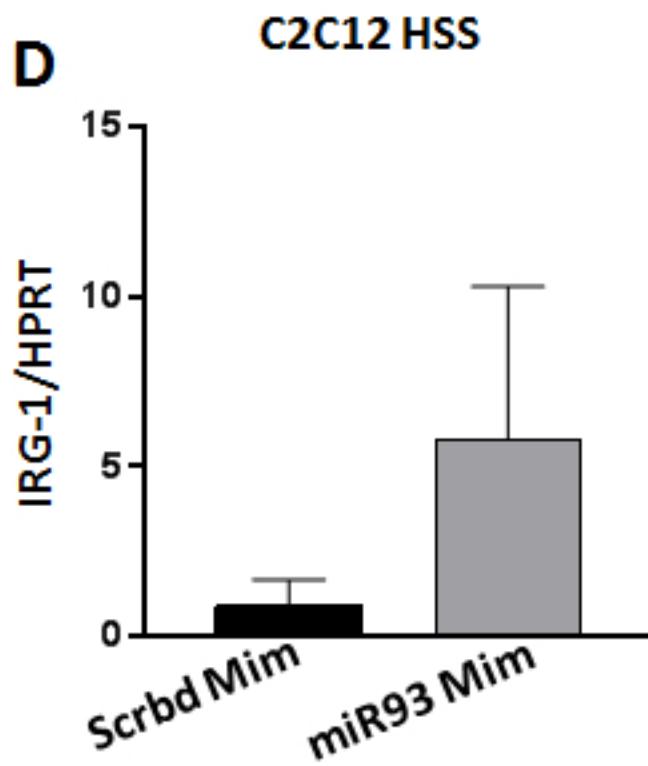
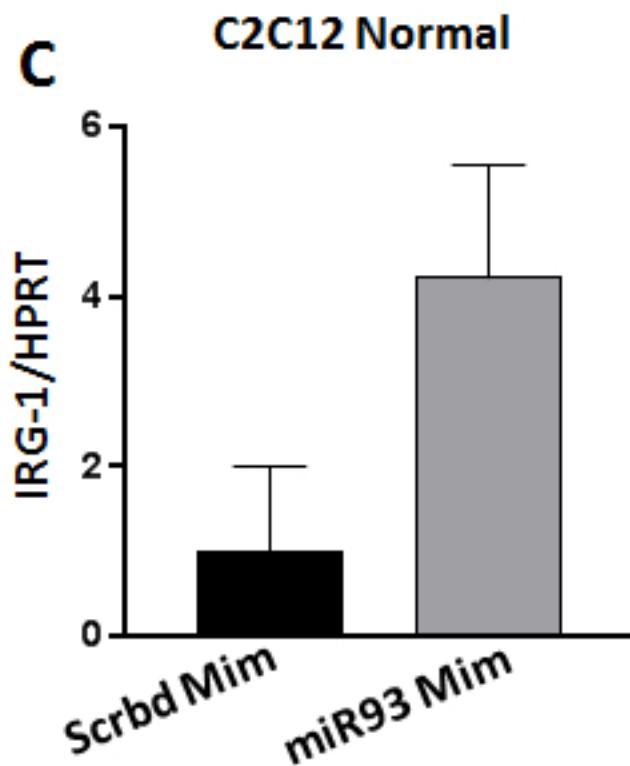
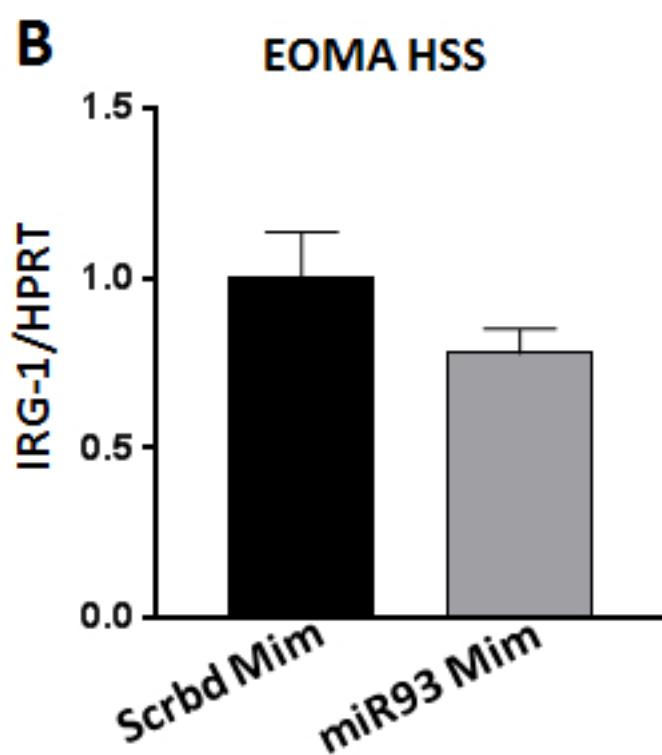
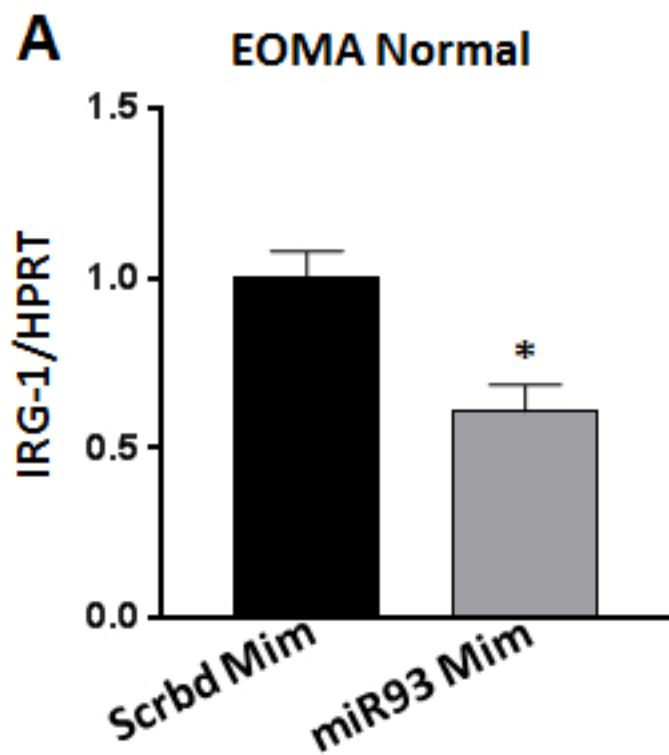
Supplemental Figure-18



Supplemental Figure-19

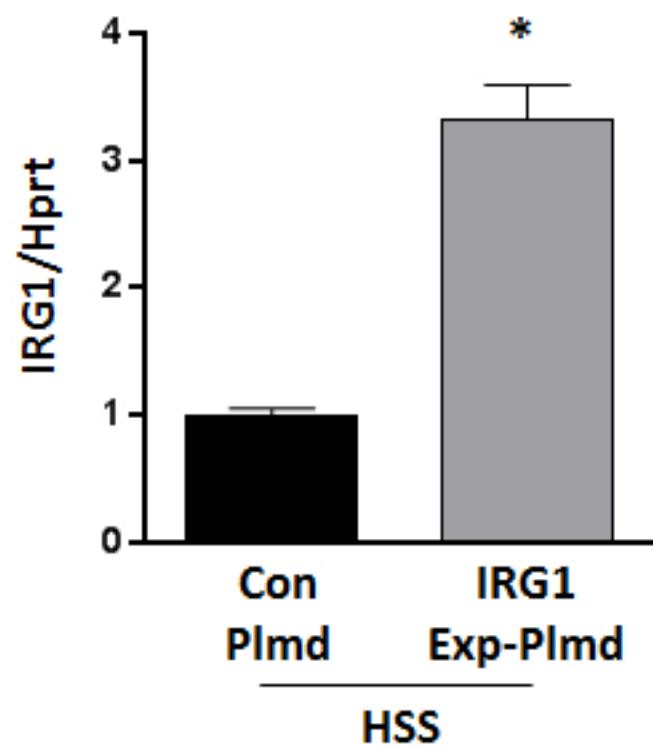
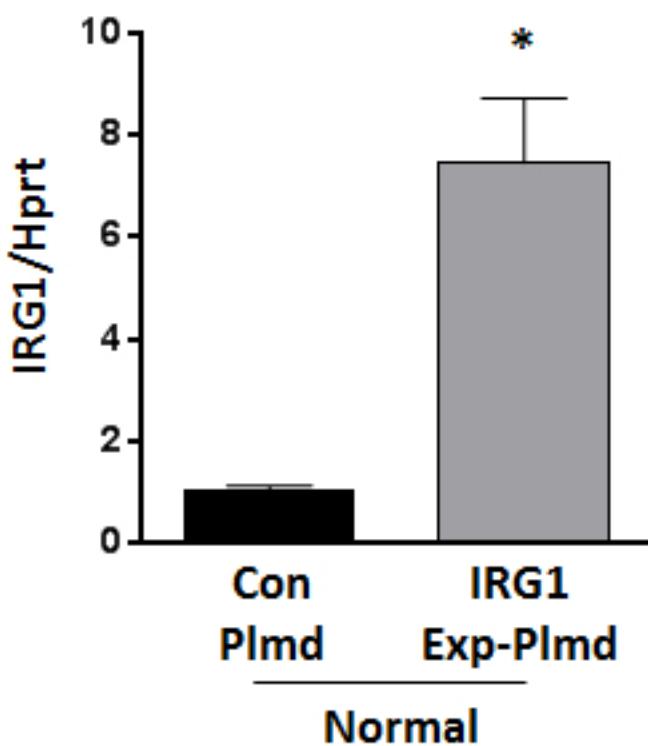


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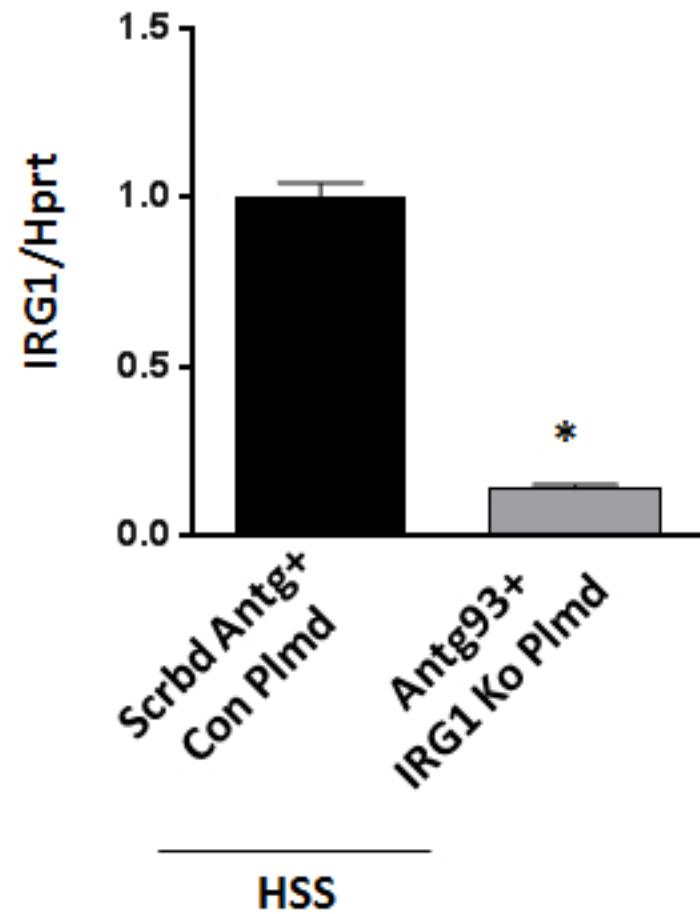
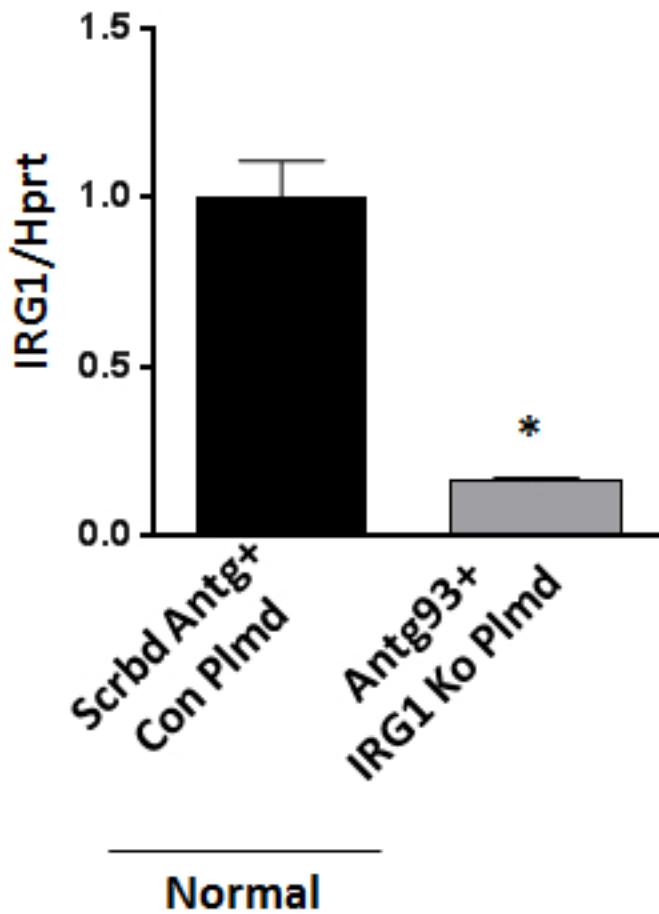


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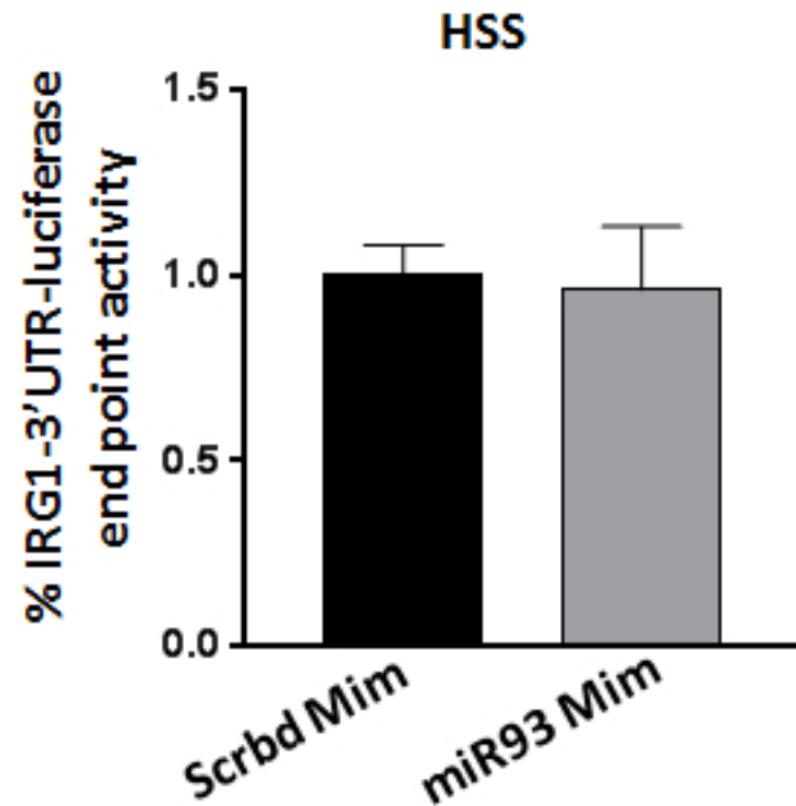
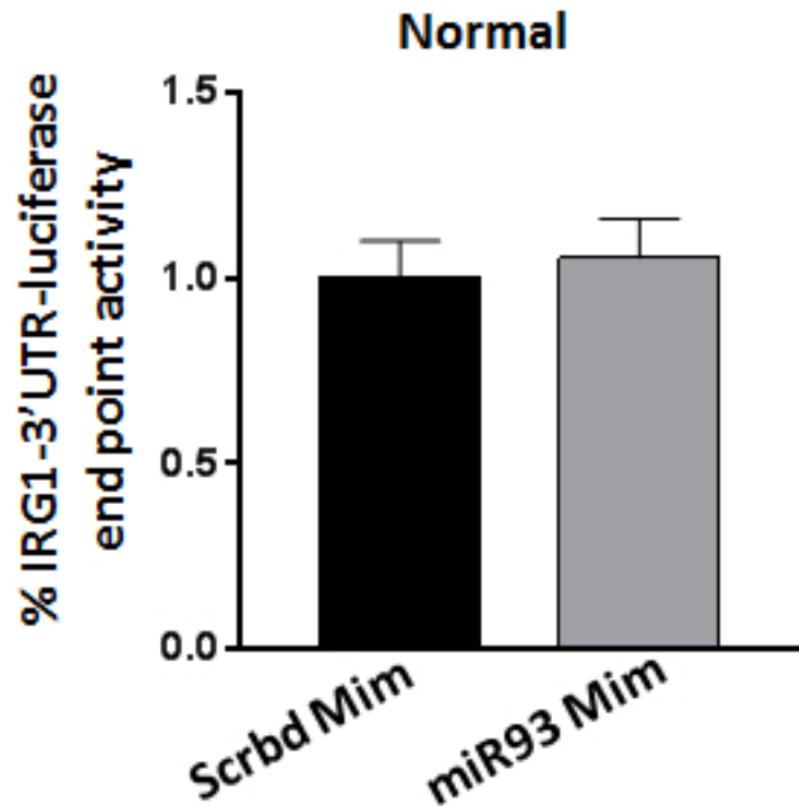
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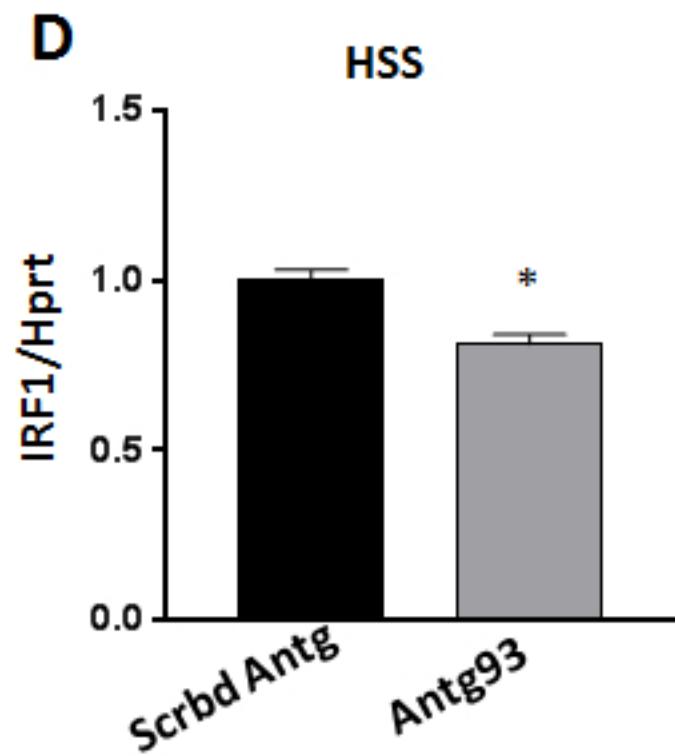
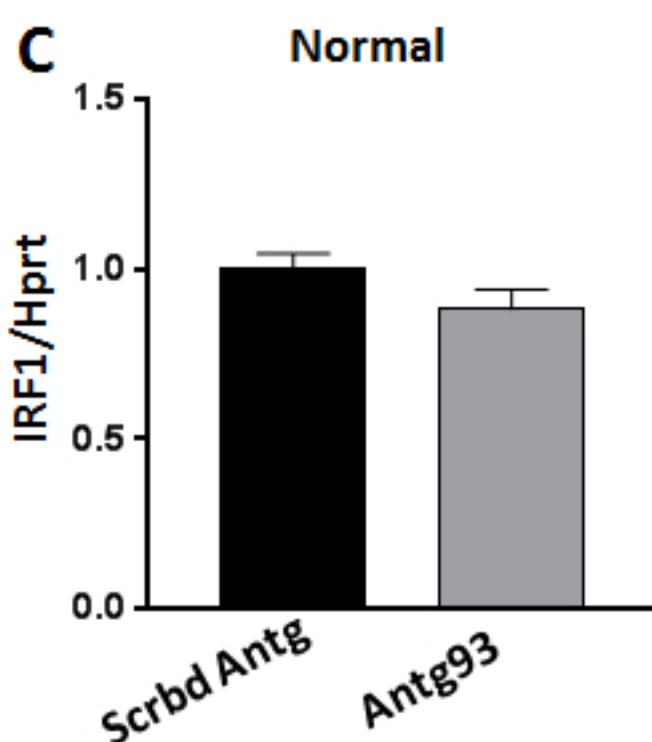
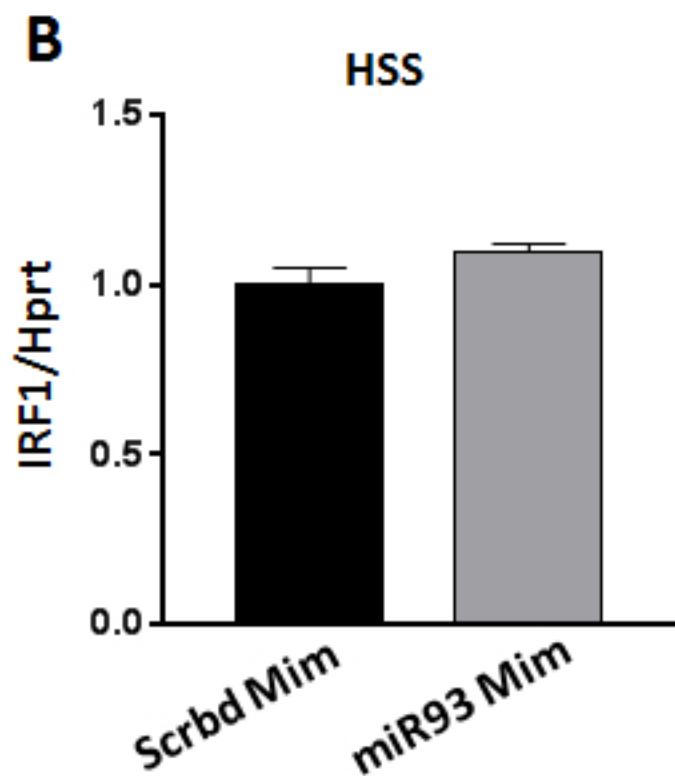
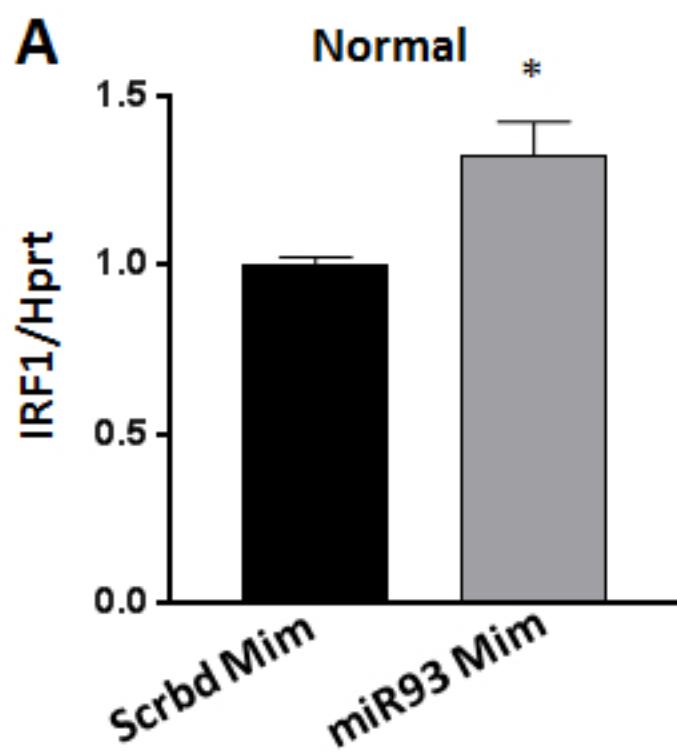
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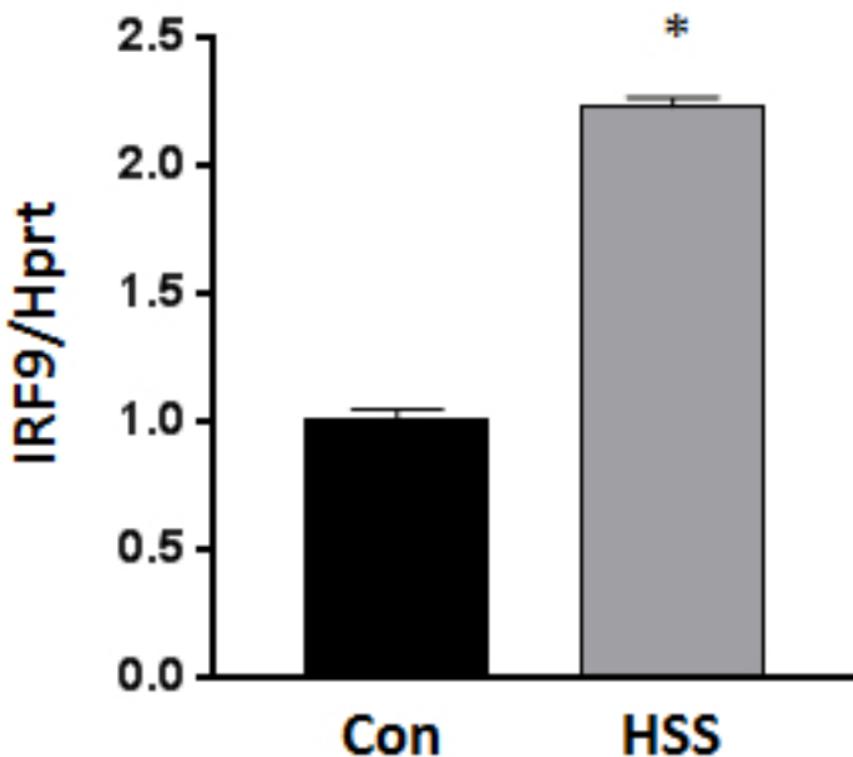
Supplemental Figure-22



Supplemental Figure-23



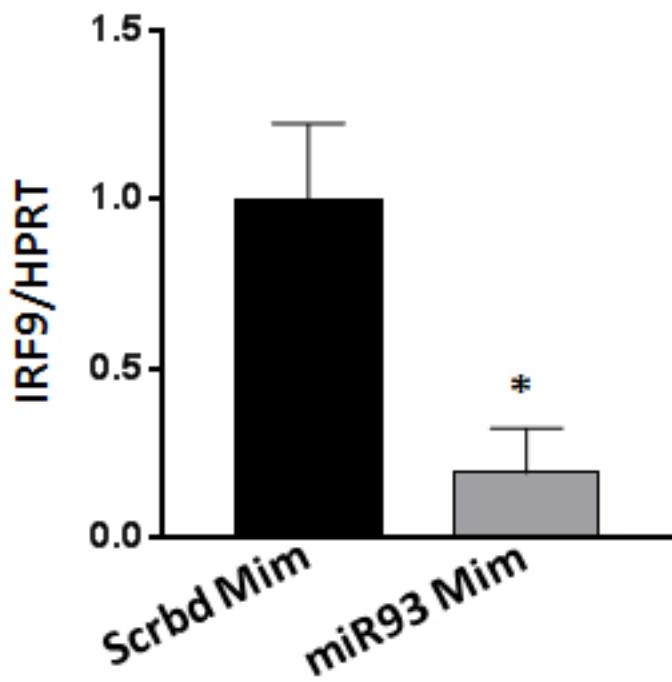
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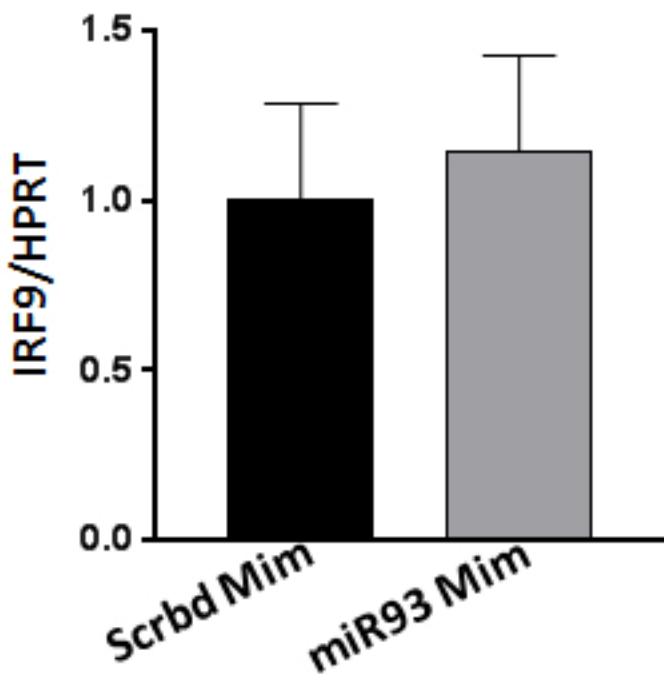
Supplemental Figure-25

A

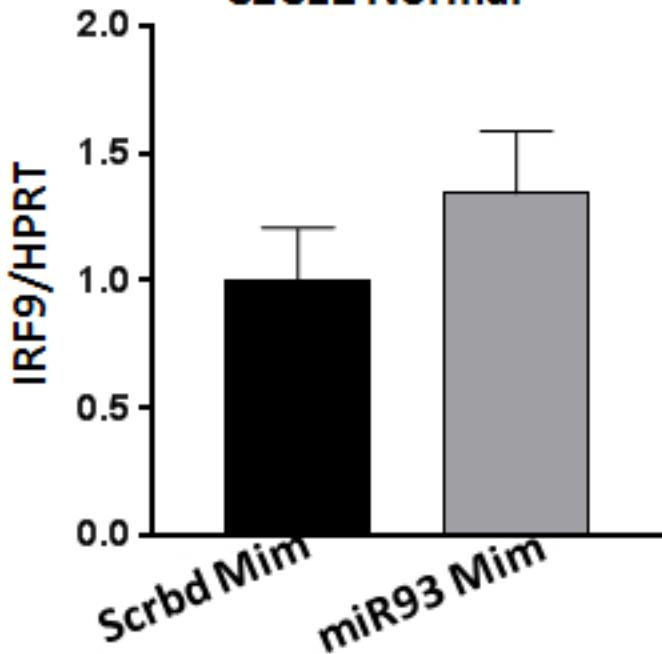
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**B**

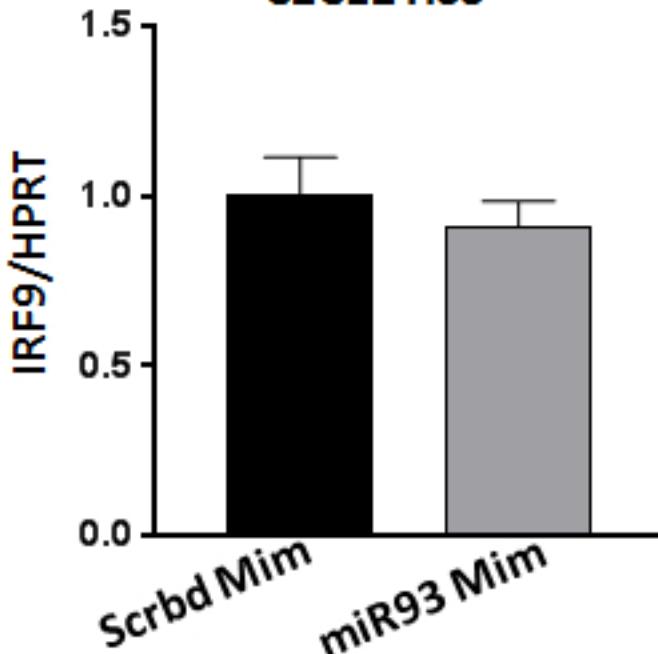
EOMA HSS

**C**

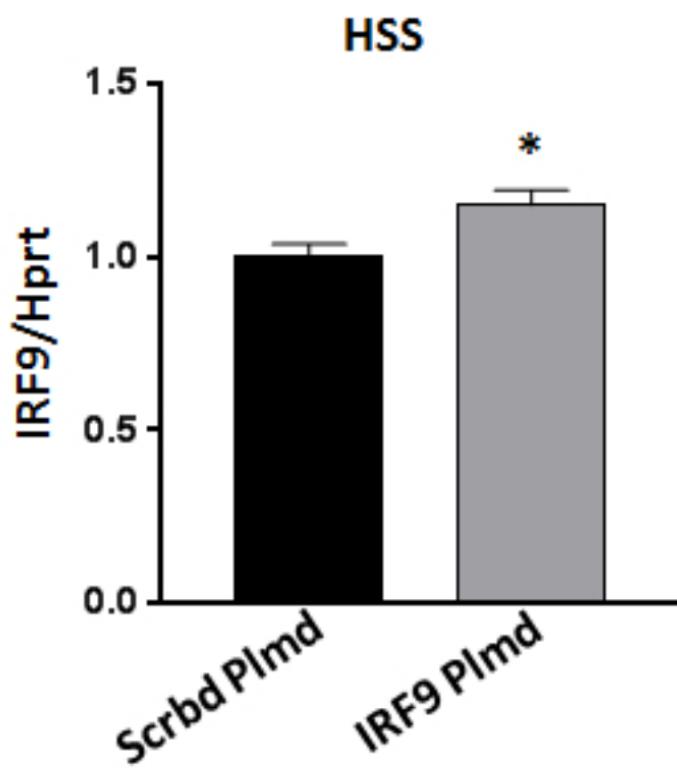
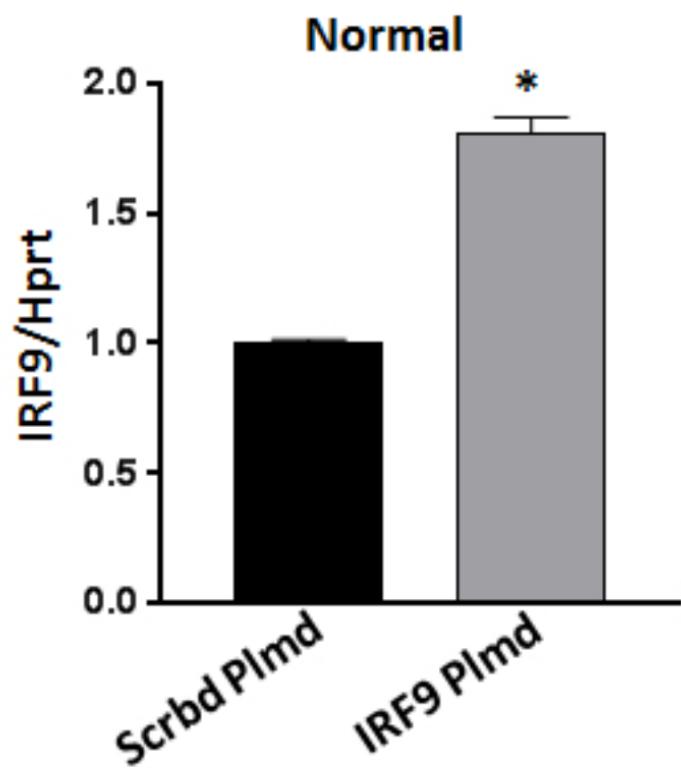
C2C12 Normal

**D**

C2C12 HSS

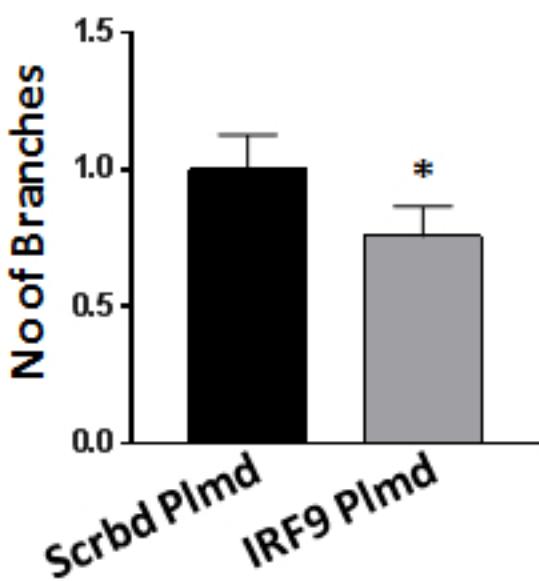
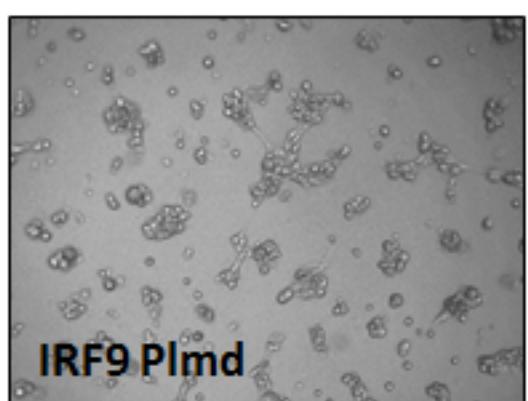
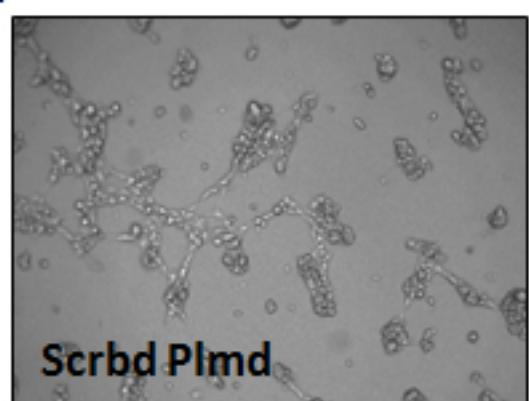


Supplemental Figure-26

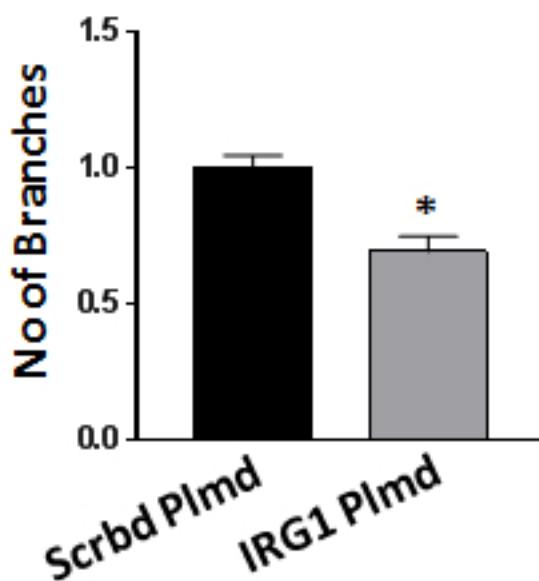
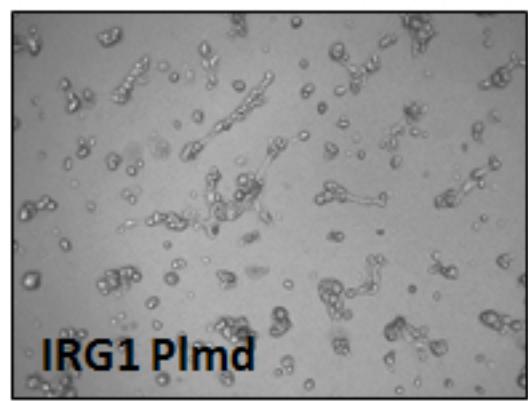
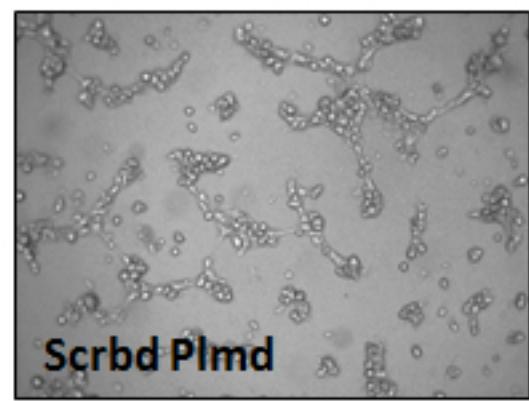


Supplemental Figure-27

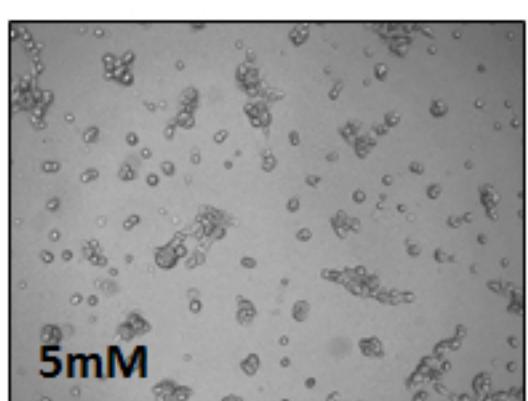
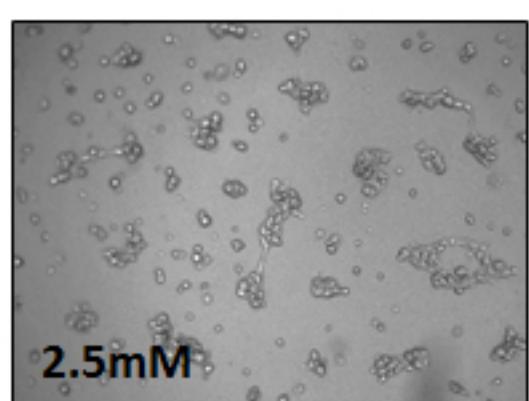
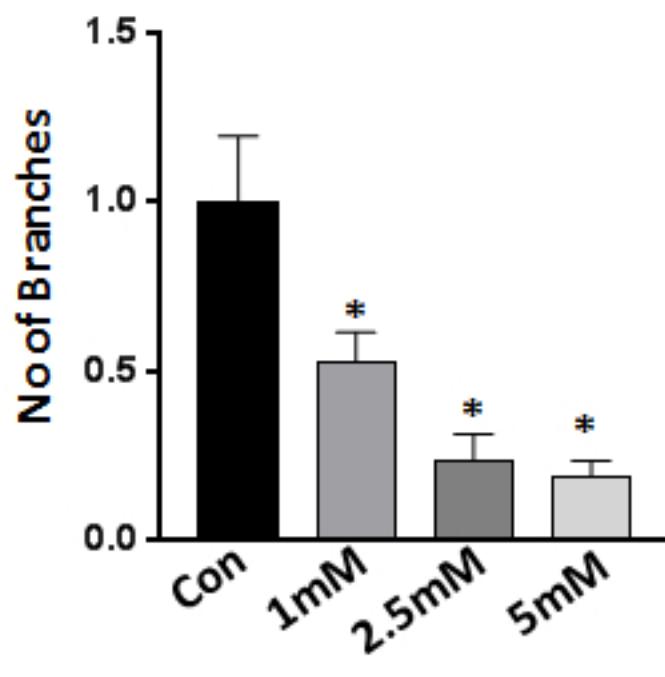
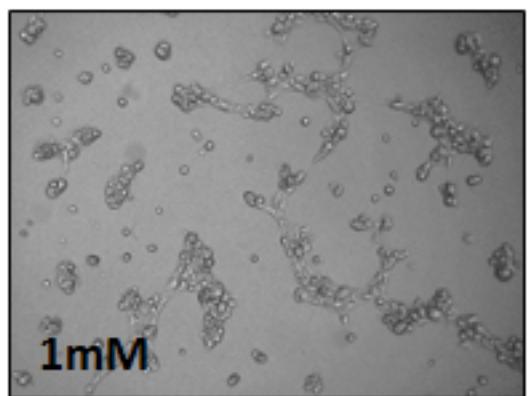
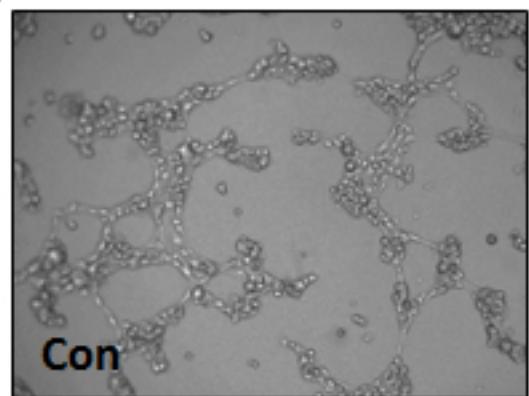
A



B



C



SUPPLEMENTAL FIGURE LEGEND

Supplemental Figure-1: qPCR analysis of miR93 expression normalized to house-keeping Sno202 in WT (wild type, C57Bl6) and miR106b-93-25^{-/-} mice skeletal muscle. n=4, P<0.05 considered significant. Unpaired T-test. Data presented as Mean±SEM.

Supplemental Figure-2: Gating strategy used in the flow cytometry experiments.

Supplemental Figure-3: Flow cytometric analysis of total, tissue infiltrating and tissue resident macrophages. A) %total live F4/80⁺ macrophages in WT and miR106b-93-25^{-/-} non-ischemic gastrocnemius-muscle (NGA). *P<0.025 considered significant. Unpaired T test with Welch's correction. B, C) %CD80^{high} ((B), M1-like-polarized) and CD206⁺ ((C), M2-like-polarized) CD11b⁺F4/80⁺ macrophages in WT and miR106b-93-25^{-/-} NGA. *P<0.025 considered significant. Unpaired T test with Welch's correction. D) % total live F4/80⁺ macrophages in WT and miR106b-93-25^{-/-} adductor-muscle from non-ischemic leg (NAM). *P<0.025 considered significant. Unpaired T test. E, F) % CD80^{high} ((E), M1-like-polarized) and CD206⁺ ((F), M2-like-polarized) CD11b⁺F4/80⁺ macrophages in WT and miR106b-93-25^{-/-} NAM. *P<0.025 considered significant. Unpaired T test. G) %infiltrating CD11b⁺F4/80⁺CX3CR1⁺ macrophages in WT and miR106b-93-25^{-/-} NGA. *P<0.025 considered significant. Unpaired T test with Welch's correction. H, I) %CD80^{high} ((H), M1-like-polarized) and CD206⁺ ((I), M2-like-polarized) CD11b⁺F4/80⁺ CX3CR1⁺ macrophages in WT and miR106b-93-25^{-/-} NGA. *P<0.025 considered significant. Unpaired T test with Welch's correction. J) %infiltrating CD11b⁺F4/80⁺CX3CR1⁺ macrophages in WT and miR106b-93-25^{-/-} NAM. *P<0.025 considered significant. Unpaired T test. K, L) %CD80^{high} ((K), M1-like-polarized) and CD206⁺ ((L), M2-like-polarized) CD11b⁺F4/80⁺CX3CR1⁺ macrophages in WT and miR106b-93-25^{-/-} NAM. *P<0.025 considered significant. Unpaired T test. M) % resident CD11b⁺F4/80⁺CX3CR1⁺ macrophages in WT and miR106b-93-25^{-/-} NGA. *P<0.025 considered significant. Unpaired T test. N, O) %CD80^{high} ((N), M1-like-polarized) and CD206⁺ ((O), M2-like-polarized) CD11b⁺F4/80⁺CX3CR1⁺ macrophages in WT and miR106b-93-25^{-/-} NGA. *P<0.025 considered significant. Unpaired T test. P) % resident CD11b⁺F4/80⁺CX3CR1⁺ macrophages in WT and miR106b-93-25^{-/-} NAM. *P<0.025 considered significant. Unpaired T test. Q, R) %CD80^{high} ((Q), M1-like-polarized) and CD206⁺ ((R), M2-like-polarized) CD11b⁺F4/80⁺ CX3CR1⁺ macrophages in WT and miR106b-93-25^{-/-} NAM. *P<0.025 considered significant. Unpaired T test. n=4. Data presented as Mean ± SEM.

Supplemental Figure-4: Representative dot plots and histograms for the flow cytometry data presented in Fig-2.

Supplemental Figure-5: Flow cytometric analysis of bone-marrow monocytes. A, D) %live total CD11b⁺CD115⁺ monocytes in WT and miR106b-93-25^{-/-} non-ischemic (A) and ischemic (D) leg bone-

marrow. *P<0.025 considered significant. Unpaired T test (Fig-4A unpaired T test with Welch's correction)). B, C, E, F) %CD80^{high} (B, E) and CD206⁺ (C, F) CD11b⁺CD115⁺ monocytes from non-ischemic (B, C) and ischemic (E, F) leg bone-marrow from WT and miR106b-93-25^{-/-} mice. *P<0.025 considered significant. n=4. Unpaired T test (4B, C-Unpaired T test with Welch's correction). Data presented as Mean ± SEM.

Supplemental Figure-6: *In vitro* angiogenesis of endothelial cells (EOMA) transfected with scrambled mimic (Scrbd Mim), miR106b mimic, miR93 mimic or miR25 mimic under normal conditions on growth factor reduced matrigel. n=4, *P<0.05 considered significant. One-way ANOVA with Dunnett's post-test.

Supplemental Figure-7: qPCR analysis A) miR93 expression in macrophages (Raw264.7) transfected with scrambled mimic (Scrbd Mim) or miR93 mimic (miR93 Mim) normalized to Sno202, B) miR106b expression in macrophages transfected with Scrbd Mim or miR106b mim normalized to Sno202, C) miR25 expression in macrophages transfected with Scrbd Mim or miR25 mim normalized to Sno202, D) miR93 expression in macrophages transfected with scrambled antagonir (Scrbd Antg) or antagonir93 (Antg93) normalized to Sno202. *P<0.05 considered significant. n=6, Unpaired T test. Data presented as Mean±SEM.

Supplemental Figure-8: A) qPCR analysis of Arg1 and iNos expression normalized to HPRT in macrophages transfected with scrambled mimimc (Scrbd Mim) or miR106b mimic (miR106b Mim) under normal conditions. n=6, Unpaired T test. B) qPCR analysis of Arg1 and iNos expression normalized to HPRT in macrophages transfected with Scrbd Mim or miR106b Mim under HSS conditions. n=6, Unpaired T test. C) qPCR analysis of Arg1 and iNos expression normalized to HPRT in macrophages transfected with Scrbd Mim or miR25 Mim under normal conditions. n=6, Unpaired T test. D) qPCR analysis of Arg1 and iNos expression normalized to HPRT in macrophages transfected with Scrbd Mim or miR25 Mim under HSS conditions. n=6, *P<0.05 considered significant. Unpaired T test. Data presented as Mean±SEM

Supplemental Figure-9: qPCR analysis of inducible nitric oxide synthase (iNos) and Arginase-1 (Arg-1) expression normalized to house-keeping Hypoxanthine Phosphoribosyltransferase (Hprt) gene expression in macrophages (Raw264.7) under HSS at various time points. n=6, Data presented as Mean±SEM.

Supplemental Figure-10: Fluorogenic detection of oxidative stress in macrophages transfected by scrambled mimic (Scrbd mim) or miR93 mimic under (A) normal and (B) HSS conditions by CellROX-green fluorescence emission. *P<0.05 considered significant. n=5. Unpaired T test. Data presented as Mean±SEM.

Supplemental Figure-11: Laser Doppler analysis of microvascular blood flow in WT-wild type (C57Bl6) HLI mice that received WT-BMDM and miR106b-93-25^{-/-} HLI mice that received miR106b-93-25^{-/-} BMDM. n=7, *P<0.05 indicates time points that are significantly different from d0 baseline perfusion within the same group by repeated measures ANOVA with Dunnetts post-test. #P<0.05 indicates the specific time points that are significantly different between 2 groups by Unpaired T-test. Data presented as Mean±SEM.

Supplemental Figure-12: qPCR analysis of miR93 expression (Ct values) in miR106b-93-25^{-/-} mice skeletal muscle transfected with scrambled plasmid (Scrbd Plmd) or miR93 expressing plasmid (miR93 Plmd). n=3. *P<0.05 is considered significant. Unpaired T-test. Data presented as Mean±SEM.

Supplemental Figure-13: Apoptosis assay by terminal deoxy uridine nick end labeling (TUNEL) in ischemic skeletal muscle. TUNEL positive cells labeled with Alexa-488 (Green) and CD31 positive cells labeled with Alexa-555 (Red) in the ischemic gastrocnemius-muscle of wild type (WT), miR106b-93-25^{-/-} mice treated with scrambled plasmid (Scrbd Plmd) or miR93 expressing plasmid (miR93 Plmd). n=5, *P<0.05 is considered significant. One way ANOVA with Dunnetts post-test. Data presented as Mean±SEM.

Supplemental Figure-14: Flow cytometric analysis of total, tissue infiltrating and tissue resident macrophages. A) %total live F4/80⁺ macrophages in non-ischemic gastrocnemius-muscle (NGA) of miR106b-93-25^{-/-} mice treated with control plasmid (con (scrambled) plmd) or miR93 expressing plasmid (miR93 Plmd)). P<0.025 considered significant. Unpaired T test. B, C) %CD80^{high} ((B), M1-like-polarized) and CD206⁺ ((C), M2-like-polarized) CD11b⁺F4/80⁺ macrophages in miR106b-93-25^{-/-} mice treated with Con or miR93 plasmid NGA. P<0.025 considered significant. Unpaired T test. D) %total live F4/80⁺ macrophages in adductor-muscle of non-ischemic leg (NAM) from miR106b-93-25^{-/-} mice treated with Con or miR93 plasmid. P<0.025 considered significant. Unpaired T test. E, F) %CD80^{high} ((E), M1-like-polarized) and CD206⁺ ((F), M2-like-polarized) CD11b⁺F4/80⁺ macrophages in miR106b-93-25^{-/-} mice treated with Con or miR93 plasmid NAM. P<0.025 considered significant. Unpaired T test. G) % infiltrating CD11b⁺F4/80⁺CX3CR1⁺ macrophages in miR106b-93-25^{-/-} mice treated with Con or miR93 plasmid NGA. P<0.025 considered significant. Unpaired T test. H, I) %CD80^{high} ((H), M1-like-polarized) and CD206⁺ ((I), M2-like-polarized) CD11b⁺F4/80⁺CX3CR1⁺ macrophages in miR106b-93-25^{-/-} mice treated with Con or miR93 plasmid NGA. P<0.025 considered significant. Unpaired T test. J) % infiltrating CD11b⁺F4/80⁺CX3CR1⁺ macrophages in miR106b-93-25^{-/-} mice treated with Con or miR93 plasmid NAM. P<0.025 considered significant. Unpaired T test. K, L) %CD80^{high} ((K), M1-like-polarized) and CD206⁺ ((L), M2-like-polarized) CD11b⁺F4/80⁺CX3CR1⁺ macrophages in miR106b-93-25^{-/-} mice treated with Con or miR93 plasmid NAM. P<0.025 considered significant. Unpaired T test. M) %resident CD11b⁺F4/80⁺

CX3CR1⁻ macrophages in miR106b-93-25^{-/-} mice treated with Con or miR93 plasmid NGA. P<0.025 considered significant. Unpaired T test. N, O) %CD80^{high} ((N), M1-like-polarized) and CD206⁺ ((O), M2-like-polarized) CD11b⁺F4/80⁺CX3CR1⁻ macrophages in miR106b-93-25^{-/-} mice treated with Con or miR93 plasmid NGA. P<0.025 considered significant. Unpaired T test. P) %resident CD11b⁺F4/80⁺CX3CR1⁻ macrophages in miR106b-93-25^{-/-} mice treated with Con or miR93 plasmid NAM. P<0.025 considered significant. Unpaired T test. Q, R) %CD80^{high} ((Q), M1-like-polarized) and CD206⁺ ((R), M2-like-polarized) CD11b⁺F4/80⁺CX3CR1⁻ macrophages in miR106b-93-25^{-/-} mice treated with Con or miR93 plasmid NAM. P<0.025 considered significant. Unpaired T test. n=4. Data presented as Mean ± SEM.

Supplemental Figure-15: Representative dot plots and histograms for the flow cytometry data presented in Fig-6.

Supplemental Figure-16: Flow cytometric analysis of bone-marrow and circulating monocytes. A, D) %live total CD11b⁺CD115⁺ monocytes from the bone-marrow of non-ischemic (A) and ischemic (D) legs from miR106b-93-25^{-/-} mice treated with scrambled (Con Plmd) or miR93-expressing plasmid (miR93 Plmd). P<0.025 considered significant. Unpaired T test (Fig-14A Unpaired T test with Welch's correction). B, C, E, F) %CD80^{high} (B, E) and CD206⁺ (C, F) CD11b⁺CD115⁺ monocytes from non-ischemic (B, C) and ischemic (E, F) leg bone-marrow from miR106b-93-25^{-/-} mice treated with Con or miR93 Plmd. P<0.025 considered significant. Unpaired T test (14B, C-Unpaired T test with Welch's correction). G) %live total CD11b⁺CD115⁺ monocytes from WT-HLI and miR106b-93-25^{-/-}-HLI blood. P<0.025 considered significant. Unpaired T test. H) %live total CD11b⁺CD115⁺ monocytes from the blood of miR106b-93-25^{-/-}-HLI mice treated with Con Plmd or miR93 Plmd. P<0.025 considered significant. Unpaired T test. I, K) %CD80^{high} (I) and CD206⁺ (K) CD11b⁺CD115⁺ monocytes from WT-HLI and miR106b-93-25^{-/-}-HLI blood. P<0.025 considered significant. Unpaired T test. J, L) %CD80^{high} (J) and CD206⁺ (L) CD11b⁺CD115⁺ monocytes in the blood of miR106b-93-25^{-/-} mice treated with Con Plmd or miR93 Plmd. P<0.025 considered significant. Unpaired T test. n=4. Data presented as Mean±SEM.

Supplemental Figure-17: (A-C) Terminal Deoxy Uridine Nick End labeling assay to detect apoptosis in (A) normal C2C12 cells treated with conditioned medium from macrophages (MΦs) transfected with scrambled antagonir (Scrbd Antg) or antagonir93 (Antg93) (B) normal C2C12 cells treated with conditioned medium from HSS macrophages transfected with scrambled mimic (Scrbd Mim) or miR93 mimic (miR93 Mim) and (C) HSS C2C12 cells treated with conditioned medium from macrophages transfected with Scrbd Mim or miR93 Mim under normal conditions. n=5. *P<0.05 considered significant. Unpaired T test. D) qPCR analysis of Arg1 and iNos expression in macrophages treated with conditioned medium from C2C12 cells transfected with Scrb Mim or miR93 Mim under normal conditions. n=6, *P<0.05 considered significant. Unpaired T test. Data presented as Mean±SEM.

Supplemental Figure-18: Cytokine/chemokine levels (pg/ml) in conditioned medium from HSS macrophages transfected with scrambled mimic or miR93 mimic by cytokine bead array. n=3, *P<0.05 considered significant. Unpaired T test between 2 specific cytokines. Data presented as Mean±SEM.

Supplemental Figure-19: A) qPCR analysis of CXCL2 expression in non-ischemic gastrocnemius-muscle (NGA) normalized to Hprt in WT (wild type, C57Bl6) and miR106b-93-25^{-/-}. n=5, *P<0.05 considered significant. Unpaired T test. B) qPCR analysis of CXCL2 expression in ischemic gastrocnemius-muscle (IGA) normalized to Hprt in WT, miR106b-93-25^{-/-} and miR106b-93-25^{-/-} mice treated with miR93 expressing plasmid. n=5, *P<0.05 considered significant. Unpaired T test. C, D) qPCR analysis of Arg-1 (C) and iNos (D) normalized to Hprt in macrophages treated with varying concentrations of CXCL2 under normal conditions. n=6, *P<0.05 considered significant. Unpaired T test. E, F) qPCR analysis of Arg-1 (E) and iNos (F) normalized to Hprt in macrophages treated with varying concentrations of CXCL2 under HSS conditions. n=6, *P<0.05 considered significant. Unpaired T test. G, H) qPCR analysis of CXCL2 expression in macrophages transfected with scrambled antagonir (Scr-Antg) or antagonir93 (Antg-93) under normal (G) or HSS (H) conditions. n=6, *P<0.05 considered significant. Unpaired T test. Data presented as Mean±SEM.

Supplemental Figure-20: (A, B) qPCR analysis of IRG1 expression in endothelial cells (EOMA) transfected with scrambled mimic (Scrbd Mim) or miR93 mimic (miR93 Mim) under (A) normal or (B) HSS conditions. qPCR analysis of IRG1 expression in endothelial cells (EOMA) transfected with scrambled mimic (Scrbd Mim) or miR93 mimic (miR93 Mim) under (A) normal or (B) HSS conditions. n=6, *P<0.05 considered significant. Unpaired T test. C, D) qPCR analysis of IRG1 expression in skeletal muscle cells (C2C12) transfected with Scrbd Mim or miR93 Mim under (C) normal or (D) HSS conditions. n=6, *P<0.05 considered significant. Unpaired T test. Data presented as Mean±SEM.

Supplemental Figure-21: A) qPCR analysis of IRG1 expression in macrophages transfected with scrambled plasmid (Con Plmd) or IRG1 expressing plasmid (IRG1 Exp-Plmd) under normal (A, Left-panel) or HSS (A, right-panel) conditions. n=6, P<0.05 considered significant. Unpaired T-test. B) qPCR analysis of IRG1 expression in macrophages transfected with scrambled antagonir (Scr-Antg) + Scrambled plasmid (Con Plmd) or antagonir 93 (Antg93) + IRG1 crispr-cas9 knock out plasmid (IRG1 KO plmd) under normal (B, left-panel) or HSS (B, right-panel) conditions. n=6, *P<0.05 considered significant. Unpaired T test. Data presented as Mean±SEM.

Supplemental Figure-22: Luciferase activity in macrophages transfected with IRF9-3'UTR-luciferase plasmid followed by transfection with scrambled mimic (Scrbd Mim) or miR93 mimic (miR93 Mim) under normal or HSS conditions. n=4, Unpaired T test. P<0.05 considered significant. Data presented as

Mean \pm SEM.

Supplemental Figure-23: (A, B) qPCR analysis of IRF1 expression in macrophages transfected with scrambled mimic (Scrbd Mim) or miR93 mimic (miR93 Mim) under (A) normal or (B) HSS conditions. n=6, *P<0.05 considered significant. Unpaired T test. C, D) qPCR analysis of IRF1 expression in macrophages (Raw264.7) transfected with scrambled antagonir (Scrbd Antg) or antagonir 93 (Antg93) under (C) normal or (D) HSS conditions. n=6, *P<0.05 considered significant. Unpaired T test. Data presented as Mean \pm SEM.

Supplemental Figure-24: qPCR analysis of IRF9 expression in macrophages (Raw264.7) under normal and HSS conditions. n=6, *P<0.05 considered significant. Unpaired T test. Data presented as Mean \pm SEM.

Supplemental Figure-25: (A, B) qPCR analysis of IRF9 expression in endothelial cells (EOMA) transfected with scrambled mimic (Scrbd Mim) or miR93 mimic (miR93 Mim) under (A) normal or (B) HSS conditions. n=6, *P<0.05 considered significant. Unpaired T test. C, D) qPCR analysis of IRF9 expression in skeletal muscle cells (C2C12) transfected with scrambled mimic (Scrbd Mim) or miR93 mimic (miR93 Mim) under (A) normal or (B) HSS conditions. n=6, Unpaired T test. Data presented as Mean \pm SEM.

Supplemental Figure-26: qPCR analysis of IRF9 expression in macrophages (Raw264.7) transfected with scrambled plasmid (Scrnd Plmd) or IRF9 expressing plasmid (Scrbd Plmd) under normal and HSS conditions. n=4. *P<0.05 considered significant. Unpaired T test. Data presented as Mean \pm SEM.

Supplemental Figure-27: *In vitro* angiogenesis assay of endothelial cells (EOMA) transfected with (A) scrambled plasmid (Scrnd Plmd) or IRF9 plasmid (IRF9 Plmd) (B) scrbd plmd or IRG1 plasmid (IRG1 Plmd) and (C) untreated, 1mM, 2.5mM or 5mM itaconid acid on growth factor reduced matrigel. n=5, Unpaired T test for 'A, B' and One way ANOVA with Dunnetts post-test for 'C'. *P<0.05 considered significant. Data presented as Mean \pm SEM.

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